

BINDING CONSTRUCTS AND METHODS FOR USE THEREOF

All applications from which this application takes priority are incorporated herein by reference in their entirety.

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FIELD OF THE INVENTION

The present invention relates generally to compounds having various utilities including uses for research, diagnostics, and therapy, for example, immunotherapy. Compounds of the invention include immunologically active proteins and protein conjugates. Such proteins include recombinant or engineered binding proteins such as, for example, binding domain-immunoglobulin fusion proteins, which may include single chain Fv-immunoglobulin fusion proteins and compounds containing single chain Fv-immunoglobulins. The present invention also relates to compositions and methods for treating conditions, diseases and disorders that would improved, eased, or lessened from the administration of, for example, polypeptide and/or nucleic acid constructs of the invention, including, for example, malignant conditions and B cell disorders, including diseases characterized by autoantibody production and/or inflammation.

BACKGROUND OF THE INVENTION

The immune system is one of the most complex of the body's many intricate systems. A vast and complicated arrangement made up of many different types of cells and involving many different kinds of molecules, the human immune system allows the body to respond to foreign invaders such as bacteria, viruses, and other infectious agents, as well as foreign material such as pollen. In general, the human immune system is divided into two main parts, antibody-mediated immunity (also called "humoral" or "circulating" immunity) and cell-mediated immunity, both of which are managed by lymphocytes. Lymphocytes are one of the five kinds of white blood cells (leukocytes) circulating in the blood. There are several kinds of lymphocytes, each with different functions to perform. The most common types of lymphocytes are B lymphocytes (B cells), which are responsible for making antibodies, and T lymphocytes (T cells). Cells of the immune system not only include T cells and B cells, but also Natural Killer Cells, granulocytes (or polymorphonuclear (PMN) leukocytes), macrophages, and dendritic cells. The humoral system is managed by B cells with help from T cells and deals with infectious agents in the

blood and tissues of the body. The cell-mediated system is managed by T cells and deals with cells of the body that have been infected.

An antigen is a substance, usually macromolecular, that stimulates or induces an immune response. Because of its complex macromolecular structure, a single microorganism consists of multiple antigens (*e.g.*, surface structures such as cell wall components, fimbriae, flagella, *etc.*, or extracellular proteins, such as toxins or enzymes produced by the microorganism). The coat proteins and some of the envelope proteins of animal viruses are also usually antigenic. A host is generally able to respond specifically to antigens that come into contact with components of its immune system. Both the antibody-mediated immunity and cell-mediated immunity systems involve complex interrelationships that allow them to mount immune reactions to almost any antigen. In other words, the immune system is able to recognize foreign substances (antigens) that stimulate the system to produce antibody-mediated immunity, cell-mediated immunity, or both.

The immune system complex is constituted by a variety of different cell types and organs disseminated throughout the body. These include the primary and secondary lymphoid organs. The primary lymphoid organs are the bone marrow and the thymus. All the cells of the immune system are initially derived from the bone marrow in a process called hematopoiesis. During hematopoiesis bone marrow-derived stem cells differentiate into either mature cells of the immune system ("B" cells) or into precursors of cells that migrate out of the bone marrow to mature in the thymus ("T" cells). In addition to red blood cells, platelets, and B cells, the bone marrow also produces Natural Killer cells, granulocytes, and immature thymocytes. The function of the thymus is to produce mature T cells. Immature thymocytes, also known as prothymocytes, leave the bone marrow and migrate into the thymus where they mature and are then released into the bloodstream. The immune system complex also includes secondary lymphoid organs, *e.g.*, the spleen, the lymph nodes, *etc.*, as well as a circulatory system that is separate from blood vessels.

The spleen, made up of B cells, T cells, macrophages, dendritic cells, Natural Killer cells, and red blood cells, is an immunologic filter of the blood. Migratory macrophages and dendritic cells capture antigens from blood that passes through the spleen. Migratory macrophages and dendritic cells also bring antigens to the spleen via the

bloodstream. An immune response is initiated in the spleen when macrophages or dendritic cells present the antigen to the appropriate B or T cells, and B cells become activated and produce large amounts of antibody.

5 Lymphatic vessels and lymph nodes are the parts of a special circulatory system that carries lymph. Lymph is a transparent fluid containing white blood cells, chiefly lymphocytes. Lymph bathes the tissues of the body, and is then collected in lymphatic vessels. Lymph nodes dot a network of lymphatic vessels and, when afferent lymph ducts bring lymph-containing antigens into the node, function as an immunologic filter for lymph. Composed mostly of T cells, B cells, dendritic cells, and macrophages, 10 the lymph nodes drain fluid from most tissues. Antigens are filtered out of the lymph in the lymph node before the lymph is returned to the circulation. Macrophages and dendritic cells that capture antigens also present these foreign materials to T and B cells in the lymph nodes, resulting in the stimulation of B cells to develop there into antibody-secreting plasma cells. Antibodies leave the lymph node by the efferent ducts that empty into the 15 blood stream. Lymphocytes can also leave the node by the efferent duct and travel to other sites in the lymphatic system or enter into the blood circulation. A single lymphocyte completes a circuit through the circulating blood and lymphatic systems once every 24 hours.

Tonsils, adenoids, Peyer's patches, and the appendix are also lymphoid 20 tissues. Peyer's patches (masses of lymphocytes) are similar to the tonsils and are found throughout the body, especially in the mucous linings of the digestive and respiratory tracts. It is the function of the phagocytic cells found in Peyer's patches and other lymphatic aggregate follicles to defend the body against, for example, inadequately digested food particles crossing the gut wall and entering the blood, and to attack unwanted 25 foreign invaders while they are still in the bowel.

The major function of B cells is the production of antibodies in response to foreign proteins of bacteria, viruses, and tumor cells. T cells are usually divided into two major groups, namely, the cytotoxic T lymphocytes ("Tc" cells or CTLs) and the helper T cells ("Th" cells or T helper cells). Th cells, also referred to as CD4+ T cells, function to 30 augment or potentiate immune responses by the secretion of specialized factors that activate other white blood cells to fight off infection. They enhance the production of antibodies by B cells. Tc cells, also called CD8+ T cells, can directly kill certain tumor

cells, viral-infected cells, and sometimes parasites. Tc cells are also important in down-regulation of immune responses. Both types of T cells often depend on the secondary lymphoid organs (the lymph nodes and spleen) as sites where activation occurs, but they are also found in other tissues of the body, including the liver, lung, blood, and intestinal and reproductive tracts.

Natural Killer cells, often referred to as NK cells, represent another type of lymphocyte and are similar to the Tc cell subset. They function as effector cells that directly kill certain tumors such as melanomas and lymphomas, and viral-infected cells. They are called "natural" killers because, unlike cytotoxic T cells, they do not need to recognize a specific antigen before carrying out their function. While NK cells, unlike the Tc cells, kill their targets without prior activation in the lymphoid organs, NK cells activated by Th cell secretions will kill tumor or viral-infected targets more effectively. NK cells target tumor cells and protect against a wide variety of infectious microbes. In several immunodeficiency diseases, including AIDS, Natural Killer cell function is abnormal. Natural Killer cells may also contribute to immunoregulation by secreting high levels of influential lymphokines.

Some NK cells have surface receptors (Fc γ RIII, also called CD16) for the Fc portion of the IgG antibody. They bind to target cells through receptors for the Fc portion of an antibody that has reacted with antigen on a target cell. This type of cell-mediated immunity is called antibody-dependent cell-mediated cytotoxicity (ADCC). NK cells may also have receptors for the C3 component of complement, another immune defense system, and therefore recognize cells that are coated with C3 as targets. ADCC is thought to be an important defense against a variety of parasitic infections caused, for example, by protozoa and helminths.

Although small lymphocytes look identical, they can be distinguished by molecules carried on their cell surface. Not only do such markers distinguish between B cells and T cells, they distinguish among various subsets of cells that behave differently. Every mature T cell, for instance, carries a marker known as T3 (or CD3). In addition, most helper T cells carry a T4 (CD4) marker, a molecule that recognizes Class II major histocompatibility complex ("MHC") antigens. A molecule known as T8 (CD8), which recognizes Class I MHC antigens, is found on many suppressor/cytotoxic T cells.

Another group of white blood cells collectively referred to as granulocytes, or polymorphonuclear leukocytes (PMNs), is composed of three cell types. These cells, neutrophils, eosinophils, and basophils are important in the removal of bacteria and parasites from the body. Neutrophils migrate through capillary walls and into infected tissue where they kill invaders (e.g., bacteria) and then engulf the remnants by phagocytosis. Eosinophils are cytotoxic, releasing the contents of their granules on an invader. Basophils leave the blood and accumulate at the site of an infection or other inflammation and discharge the contents of their granules, releasing a variety of mediators such as histamine, serotonin, prostaglandins and leukotrienes that, for example, increase blood flow to the area. Mediators released by basophils also play an important part in some allergic responses such as hay fever and anaphylactic responses to insect stings.

Monocytes are large phagocytic white blood cells released from the bone marrow into the blood circulation. When a monocyte enters tissue, it develops into a macrophage. Macrophages are also large, phagocytic cells that engulf foreign material (antigens) that enter the body, as well as dead and dying cells of the body. Macrophages are important in the regulation of immune responses, and are often referred to as scavengers, or antigen-presenting cells (APCs) because they pick up and ingest foreign materials and present these antigens to other cells of the immune system such as T cells and B cells. This is one of the important first steps in the initiation of an immune response. Stimulated macrophages exhibit increased levels of phagocytosis and also secrete Interleukin-1 (IL-1), a product that helps to activate B cells and T cells.

Dendritic cells also originate in the bone marrow and function as APCs. They are usually found in the structural compartment of lymphoid organs such as the thymus, lymph nodes and spleen, but are also found in the bloodstream and other tissues. It is believed that dendritic cells capture antigen or bring it to the lymphoid organs where an immune response is initiated.

Important features of the immunological system relevant to host defense and/or immunity to pathogenic microorganisms include specificity, memory, and tolerance. It is understood, for example, that an antibody or reactive T cell will react specifically with the antigen that induced its formation; it will not react with other antigens. Generally, this specificity is of the same order as that of enzyme-substrate specificity or receptor-ligand specificity, although cross-reactivity is possible. The specificity of the immune response is

explained by clonal selection. During the primary immune response, a specific antigen selects a pre-existing clone of specific lymphocytes and stimulates its activation, proliferation and differentiation. It is also understood that once the immune system has responded to produce a specific type of antibody or reactive T cell, it is capable of producing more of the antibody or activated T cell more rapidly and in larger amounts; this is called the secondary (or memory) response. It is also recognized that an animal generally does not undergo an immunological response to its own (potentially-antigenic) components. The animal is said to be tolerant, or unable to react to its own potentially antigenic components. This ensures that under normal conditions, an immune response to “self” antigens (called an autoimmune response) does not occur. Tolerance is brought about in a number of ways, but in essence the immune system is able to distinguish “self” components from “non-self” (foreign) antigens; it will respond to “non-self” but not to “self”. Sometimes in an animal, tolerance can be “broken”, which may result in an autoimmune disease.

The biological activities of the antibody-mediated and cell-mediated immune responses are different and vary from one type of infection to another. There are several classes or types of antibodies (and subclasses of various types) involved in antibody-mediated immunity. All of the classes of antibodies that are produced in response to a specific antigen react stereochemically with that antigen and not with other (different) antigens. The host has the genetic capacity to produce specific antibodies to thousands of different antigens, but does not do so until there is an appropriate (specific) antigenic stimulus. Due to clonal selection, the host produces only the homologous antibodies that will react with that antigen which, as noted above, are found in blood (plasma), lymph, and many extravascular tissues. Once the antibody-mediated immunity response occurs following interaction of B lymphocytes with antigen and their differentiation into antibody-secreting plasma cells, the secreted antibody binds to the antigen which, in turn, results in its neutralization or elimination from the body.

Cell-mediated immunity, on the other hand, is mediated by specific subpopulations of T-lymphocytes called effector T cells that exist in precursor form as “resting T cells” (pT cells). These cells bear receptors for specific antigens and recognize these antigens on the surfaces of other cells. Stimulation with that antigen results in T cell activation. T cells enlarge, enter into a mitotic cycle, reproduce and develop into effector T

cells whose activities are responsible for this type of immunity. They also develop into clones of identical reactive T cells called memory T cells. As noted above, most of the T cells in the body belong to one of two subsets and are distinguished by the presence on their surface of one or the other of two glycoproteins designated CD4 and CD8. Which of these molecules is present determines the types of cells to which the T cell can bind. T cells bearing CD8 (CD8⁺ T cells) always recognize antigen in association with Class I MHC proteins and typically function as cytotoxic T cells. Almost all the cells of the body express Class I MHC molecules. T cells bearing CD4 (CD4⁺ T cells) always recognize antigens in association with Class II MHC proteins on the surfaces of other cells. Only specialized antigen-presenting cells express Class II MHC molecules, including dendritic cells, phagocytic cells such as macrophages, and B cells. CD4⁺ T lymphocytes generally function as T helper cells.

T helper cells, which include Th1 cells and Th2 cells, respond to antigen with the production of lymphokines. Th1 and Th2 cells can be distinguished based on their lymphokine profiles. Like all T cells, Th cells arise in the thymus. When they are presented with an antigen by antigen-presenting dendritic cells they begin to proliferate and become activated. There are two kinds of dendritic cell, DC1 cells (descended from monocytes) and DC2 cells (which appear to be derived from lymphocytes).

Th1 cells (inflammatory Th1 cells involved in the elimination of pathogens residing intracellularly in vesicular compartments) are produced when DC1-type dendritic cells present antigen to the T cell receptor for antigen (TCR) and secrete Interleukin 12 (IL-12). This paracrine stimulation activates Th1 cells to secrete their own lymphokines, in particular, Tumor-Necrosis Factor-beta (TNF- β) (also known as lymphotoxin) and Interferon-gamma (IFN- γ). These lymphokines stimulate macrophages to kill bacteria they have engulfed by phagocytosis and they recruit other leukocytes to the site producing inflammation. Th1 cells are essential for cell-mediated immunity and for controlling intracellular pathogens such as, for example, *Listeria* and *Mycobacterium tuberculosis*.

Th2 cells ("true" helper Th2 cells, which are required for antibody production by B cells) are produced when DC2-type dendritic cells present antigen to the T cell receptor for antigen and, presumably, one or more paracrine stimulants. The major lymphokines secreted by Th2 cells are Interleukin 4 (IL-4), which stimulates class-switching in B cells and promotes their synthesis of IgE antibodies, acts as a positive-

feedback device promoting more pre-Th cells to enter the Th2 pathway, and blocks expression of the IL-12 receptor thereby inhibiting pre-Th cells in the thymus from entering the Th1 pathway. IL-4 also causes B cells to proliferate and differentiate into antibody-secreting plasma cells and memory B cells. IL-4 activates only B cells in the vicinity which themselves have bound the antigen, and not others, so as to sustain the specificity of the immune response. Th2 cells also produce Interleukin 5 (IL-5, which attracts and activates eosinophils), Interleukin 10 (IL-10, which inhibits IL-12 production by DCs and prevents maturation of pre-Th cells to Th1 cells), and Interleukin 13 (IL-13, which also promotes the synthesis of IgE antibodies).

Activation of the Th2 cell also causes it to begin to produce Interleukin 2 (IL-2), and to express a membrane receptor for IL-2. The secreted IL-2 autostimulates proliferation of Th2 cells. For example, IL-2 binds to IL-2 receptors on other T cells (which have bound the antigen) and stimulates their proliferation. In addition to IL-2, stimulated Th2 cells also produce IFN- γ and Interleukin 6 (IL-6), which mediate various aspects of the immune response. IFN- γ activates Natural Killer cells to their full cytolytic potential, and is an activator of macrophages and thus increases their antitumor activities. If the macrophages are infected by intracellular parasites, it activates macrophages, which in turn destroy the parasites. IFN- γ also reinforces the antitumor activities of the cytotoxic lymphocytes, increases the nonspecific activities of NK-cells, and is one of the factors that controls the differentiation of B cells and increases the secretion of immunoglobins. IL-6 stimulates several types of leukocytes, as well as the production of Acute Phase Proteins in the liver. It is particularly important in inducing B cells to differentiate into antibody forming (plasma) cells. Thus, Th2 cells provide help for B cells and are essential for antibody-mediated immunity.

Cytotoxic T lymphocytes are able to kill cells that show a new or foreign antigen on their surface (for example, virus-infected cells, or tumor cells, or transplanted tissue cells). The CD8⁺ CTLs also come in two subsets: Tc1 that, like Th1 cells, secrete IFN- γ , and Tc2 that, like Th2 cells, secrete IL-4.

The cell-mediated immunity response also plays a role in destruction of tumor cells and in rejection of tissue transplants in animals. A major problem in tissue transplantation is rejection, which is often based on cell-mediated immunity response to "foreign" cells (because they are not a perfect antigenic match). Because tumor cells

contain specific antigens not seen on normal cells they also may be recognized as foreign and destroyed by the forces of cell-mediated immunity. If tumor cells develop on a regular basis in animals, it may be cell-mediated immunity that eliminates them or holds them in check. The increase in the incidence of many types of cancer (tumors) in humans with
5 advancement of age may be correlated with a decline in the peak efficiency of the immune system that occurs about 25 years of age.

A summary of the types of cells involved in the expression of cell-mediated immunity follows. Tc lymphocytes kill cells bearing foreign antigen on surface in association with Class I MHC and can kill cells that are harboring intracellular parasites
10 (either bacteria or viruses) as long as the infected cell is displaying a microbial antigen on its surface. Tc cells kill tumor cells and account for rejection of transplanted cells. Tc cells recognize antigen-Class I MHC complexes on target cells, contact them, and release the contents of granules directly into the target cell membrane that lyses the cell. Th lymphocytes produce lymphokines that are "helper" factors for development of B cells into
15 antibody-secreting plasma cells. They also produce certain lymphokines that stimulate the differentiation of effector T lymphocytes and the activity of macrophages. Th1 cells recognize antigen on macrophages in association with Class II MHC and become activated (by IL-1) to produce lymphokines including IFN- γ that activates macrophages and NK cells. These cells mediate various aspects of the cell-mediated immunity response
20 including delayed-type hypersensitivity reactions. Th2 cells recognize antigen in association with Class II MHC on an APC and then produce interleukins and other substances that stimulate specific B cell and T cell proliferation and activity. Macrophages are important as antigen-presenting cells that initiate T cell interactions, development, and proliferation. Macrophages are also involved in expression of cell-mediated immunity
25 because they become activated by IFN- γ produced in a cell-mediated immunity response. Activated macrophages have increased phagocytic potential and release soluble substances that cause inflammation and destroy many bacteria and other cells. Natural Killer cells are cytotoxic cells that lyse cells bearing new antigen regardless of their MHC type and even lyse some cells that bear no MHC proteins. NK cells are defined by their ability to kill
30 cells displaying a foreign antigen (*e.g.*, tumor cells) regardless of MHC type and regardless of previous sensitization (exposure) to the antigen. NK cells can be activated by IL-2 and IFN- γ , and lyse cells in the same manner as cytotoxic T lymphocytes. Some NK cells have

receptors for the Fc domain of the IgG antibody and are thus able to bind to the Fc portion of IgG on the surface of a target cell and release cytolytic components that kill the target cell via antibody-dependent cell-mediated cytotoxicity.

Extracellular factors that affect cell proliferation and differentiation have
5 been defined as cytokines. These include the lymphokines, which are proteins produced by T-lymphocytes that have effects on the differentiation, proliferation, and activity of various cells involved in the expression of cell-mediated immunity. In general, lymphokines function by (1) focusing circulating leukocytes and lymphocytes into the site of immunological encounter; (2) stimulating the development and proliferation of B cells and
10 T cells; (3) stimulating and preparing macrophages for their phagocytic tasks; (4) stimulating Natural Killer cells; and (5) providing antiviral cover and activity. A summary of various important lymphokines follows. Initially referred to as lymphocyte activation factor, IL-1 is mainly a product of macrophages, and has a variety of effects on various types of cells. It acts as a growth regulator of T cells and B cells, and it induces other cells
15 such as hepatocytes to produce proteins relevant to host defense. IL-1 forms a chemotactic gradient for neutrophils and serves as an endogenous pyrogen that produces fever. Thus, IL-1 plays an important role in both the immune responses and in the inflammatory response. IL-2 stimulates the proliferation of T cells and activates NK cells. IL-3 regulates the proliferation of stem cells and the differentiation of mast cells. IL-4 causes B
20 cell proliferation and enhanced antibody synthesis. IL-6 (also referred to as Interferon-beta2, hybridoma growth factor, B-cell differentiation factor, and hepatocyte stimulatory factor) has effects on B cell differentiation and on antibody production and on T cell activation, growth, and differentiation, and probably has a major role in the mediation of the inflammatory and immune responses initiated by infection or injury. IL-8 is a
25 chemotactic attractant for neutrophils. IL-13 shares many of the properties of IL-4, and is a potent regulator of inflammatory and immune responses. IFN- γ is produced by T cells and may be considered a lymphokine. It is sometimes called "immune interferon" (Interferon-alpha being referred to as "leukocyte interferon" and Interferon-beta being referred to as "fibroblast interferon"). IFN- γ has several antiviral effects including
30 inhibition of viral protein synthesis in infected cells. It also activates macrophages and NK cells, and stimulates IL-1, IL-2, and antibody production. Lymphotoxins include the Tumor Necrosis Factors. T cells produce TNF-beta, while TNF-alpha is produced by T

cells as well as other types of cells. TNFs function to kill cells, including tumor cells (at a distance). There are several Colony Stimulating Factors (CSFs), including granulocyte macrophage colony stimulating factor (GM-CSF), which cause phagocytic white cells of all types to differentiate and divide.

5 The nature of the membrane receptors for antigen on B cells and T cells is fairly well understood. Each B cell has approximately 10^5 membrane-bound antibody molecules (IgD or IgM) that correspond in specificity to the antibody the cell is programmed to produce (these receptors being referred to as BCRs). CD32 (Fc γ RII) on B cells are receptors for the Fc region of IgG. CD21 and CD35 on B cells are receptors for
10 complement components. Each T cell has about 10^5 molecules of a specific antigen-binding T cell receptor (a TCR) exposed on its surface. The TCR is similar, but not identical, to an antibody. There are two types of T cells that differ in their TCRs, alpha/beta ($\alpha\beta$) T cells and gamma/delta ($\gamma\delta$) T cells. The TCR of alpha/beta T cells binds a bimolecular complex displayed by a Class I MHC molecule at the surface of an antigen-
15 presenting cell. As noted above, most Th cells express CD4, whereas most Tc cells express CD8.

Both BCRs and TCRs are similar in that they are integral membrane proteins, they are present in thousands of identical copies exposed at the cell surface, they are made before the cell ever encounters an antigen, they are encoded by genes assembled
20 by the recombination of segments of DNA, they have a unique binding site that binds through non-covalent forces to a portion of the antigen called an epitope (or antigenic determinant) that depends on complementarity of the surface of the receptor and the surface of the epitope, and successful binding of the antigen receptor to the epitope, if accompanied by additional signals, results in stimulation of the cell to leave G_0 and enter
25 the cell cycle and repeated mitosis that leads to the development of a clone of cells bearing the same antigen receptor, *i.e.*, a clone of cells of the identical specificity. BCRs and TCRs differ in their structure, the genes that encode them, and the type of epitope to which they bind.

Induction of a primary immune response begins when an antigen penetrates
30 epithelial surfaces. It will eventually come into contact with macrophages or certain other classes of antigen presenting cells, including B cells, monocytes, dendritic cells, Langerhans cells, and endothelial cells. Antigens, such as bacterial cells, are internalized

by endocytosis and "processed" by APCs, then "presented" to immunocompetent lymphocytes to initiate the early steps of the immunological response. Processing by a macrophage (for example) results in attaching antigenic materials to the surface of the membrane in association with Class II MHC molecules on the surface of the cell. The antigen-class II MHC complex is presented to a T-helper (Th2) cell, which is able to recognize processed antigen associated with a Class II MHC molecule on the membrane of the macrophage. This interaction, together with stimulation by IL-1 from secreted by the macrophage, will activate the Th2 cell.

As indicated above, B cells themselves behave as APCs. Cross-linked antigens bound to antibody receptors on the surface of a B cell cause internalization of some of the antigen and expression on the B cell membrane together with Class II MHC molecules. The Th2 cell recognizes the antigen together with the Class II MHC molecules, and secretes the various lymphokines that activate the B cells to become antibody-secreting plasma cells and memory B cells. Even if the antigen cannot cross-link the receptor, it may be endocytosed by the B cell, processed, and returned to the surface in association with Class II MHC where it can be recognized by specific Th2 cells which will become activated to initiate B cell differentiation and proliferation. In any case, the overall B cell response leads to antibody-mediated immunity.

The antigen receptors on B cell surfaces are thought to be the specific types of antibodies that they are genetically programmed to produce. Hence, there are thousands of sub-populations of B cells distinguished by their ability to produce a unique antibody molecule. B cells can also react with a homologous antigen on the surface of the macrophage, or with soluble antigens. When a B cell is bound to antigen, and simultaneously is stimulated by IL-4 produced by a nearby Th2 cell, the B cell is stimulated to grow and divide to form a clone of identical B cells, each capable of producing identical antibody molecules. The activated B cells further differentiate into plasma cells which synthesize and secrete large amounts of antibody, and into memory B cells. The antibodies produced and secreted by the plasma cells will react specifically with the homologous antigen that induced their formation. Many of these reactions lead to host defense and to prevention of reinfection by pathogens. Memory cells play a role in secondary immune responses. Plasma cells are relatively short-lived (about one week) but produce large amounts of antibody during this period. Memory cells, on the other hand,

are relatively long-lived and upon subsequent exposure to antigen they become quickly transformed into antibody-producing plasma cells.

Generation of cell mediated immunity begins when, for example, a cytotoxic T cell recognizes a processed antigen associated with Class I MHC on the membrane of a cell (usually an altered self cell, but possibly a transplanted tissue cell, or a eukaryotic parasite). Under stimulation by IL-2 produced by Th2 cells, the Tc cell becomes activated to become a cytotoxic T lymphocyte capable of lysing the cell which is showing the new foreign antigen on its surface, a primary manifestation of cell-mediated immunity. The interaction between an antigen-presenting macrophage and a Th cell stimulates the macrophage to produce and secrete a Interleukin-1 that acts locally on the Th cell, stimulating the Th-cell to differentiate and produce its own cytokines (which may here be called lymphokines because they arise from a lymphocyte). These lymphokines have various functions. IL-4 has an immediate effect on nearby B cells. IL-2 has an immediate effect on T cells as described above.

Leucocytes also express adhesion-promoting receptors that mediate cell-cell and cell-matrix interactions. These adhesive interactions are crucial to the regulation of haemopoiesis and thymocyte maturation, the direction and control of leucocyte traffic and migration through tissues, and the development of immune and non-immune inflammatory responses. Several families of adhesion receptors have been identified. The leucocyte integrin family comprises three alpha-beta heterodimeric membrane glycoproteins that share a common beta subunit, designated CD18. The alpha subunits of each of the three members, lymphocyte function associated antigen-1 (LFA-1), macrophage antigen-1 (Mac-1) and p150, are designated CD11a, b, and c respectively. These adhesion molecules play a critical part in the immune and inflammatory responses of leucocytes. The leucocyte integrin family is, in turn, part of the integrin superfamily, members of which are evolutionally, structurally and functionally related. Another integrin subfamily found on leucocytes is the VLA group, so-called because the "very late activation antigens" VLA-1 and VLA-2 were originally found to appear late in T-cell activation. Members of this family function mainly as extracellular matrix adhesion receptors and are found both on haemopoietic and non-haemopoietic cells. They play a part in diverse cellular functions including tissue organization, lymphocyte recirculation and T-cell immune responses. Another integrin subfamily, the cytoadhesins, are receptors on platelets and endothelial

cells that bind extracellular matrix proteins. A second family of adhesion receptors is the immunoglobulin superfamily, members of which include CD2, LFA-3, and ICAM-1, which participate in T-cell adhesive interactions, and the antigen-specific receptors of T and B cells, CD4, CD8, and the MHC Class I and II molecules. Another recognized family
5 of adhesion receptors is the selectins, characterized by a common lectin domain. Leucocyte adhesion molecule-1 (LAM-1), which is the human homologue of the murine homing receptor, MEL-14, is expressed on leucocytes, while endothelial leucocyte adhesion molecule-1 (ELAM-1) and granule membrane protein (GMP-140) are expressed on stimulated endothelial cells and activated platelets.

10 Activation of an immune response requires physical cell-cell contact in addition to cytokines. Thus, for example, development of B and T cell precursors require intimate contact with stromal cells. At least three critical cell-cell contact events are required for the generation of immune responses. The first is initial contact of a specific antigen with a naive T cell. Because of the requirement for MHC presentation, this is an
15 obligate cell contact event. In normal situations the critical antigen presenting cell is the dendritic cell. In addition to the MHC/peptide-TCR interaction there are other non-antigen specific membrane bound ligand-receptor pairs that are important for the dendritic cell-T cell interaction. The principal one is the association of the CD28 molecule on the T cell with either of two ligands, B7.1 (CD80) and B7.2 (CD86), on the dendritic cell. These
20 molecules are termed accessory molecules and it is understood that the CD28 molecule delivers an essential second signal to the T cell without which the T cell does not become activated.

A second essential cell-cell contact is between the activated T cell and an antigen-specific B cell. Most antigens are T cell-dependent, that is, an antibody response
25 to the antigen absolutely requires T cell help. This help is delivered both by cytokines and by cell-cell contact. Cells bind specific antigen via surface Ig, then internalize, process, and present it on Class II MHC molecules. This enables them to be recognized by T cells specific for helper epitopes from the specific antigen. This cell-cell interaction also requires CD28 binding to B7 on the B cell. In addition, a molecule called CD40 ligand or
30 CD154, the expression of which is induced upon T cell activation, binds to CD40 on B cells. CD40 crosslinking promotes B cell proliferation, prevents apoptosis of germinal-center B cells, and promotes immunoglobulin isotype switching. The CD28-B7 and CD40-

CD40L receptor ligand interactions are both essential for the dialogue between B and T cells that causes their mutual activation.

A third cell-cell interaction that is essential in immune responses is the binding of activated B cells (which have migrated into a specialized structure in lymphoid organs called germinal centers) to follicular dendritic cells (FDCs). FDCs are specialized stromal cells that hold intact, *i.e.*, unprocessed, antigen on their surface in the form of long-lived immune complexes. Among other molecules, FDCs express CD23, which binds to germinal center B cells via a CR2 receptor and stimulates differentiation to plasma cells.

Time is required before a primary immune response is effective as a host defense. Antigens have to be recognized, taken up, digested, processed, and presented by APCs. A few select Th cells must react with antigen and respond; preexisting B or T lymphocytes must encounter the antigen and proliferate and differentiate into effector cells (plasma cells or Tc cells). In the case of antibody-mediated immunity, antibody level has to build up to an effective physiological concentration to render its host resistant. It may take several days or weeks to reach a level of effective immunity, even though this immunity may persist for many months, or years, or even a lifetime, due to the presence of the antibodies. In natural infections, the inoculum is small, and even though the antigenic stimulus increases during microbial replication, only small amounts of antibody are formed within the first few days, and circulating antibody is not detectable until about a week after infection.

With regard to induction of a secondary immune response, it is understood that on re-exposure to microbial antigens (secondary exposure to antigen), there is an accelerated immunological response, *i.e.*, the secondary or memory response. Larger amounts of antibodies are formed in only 1-2 days. This is due to the activities of specific memory B cells or memory T cells which were formed during the primary immune response. These memory cells, when stimulated by homologous antigen, "remember" having previously seen the antigen, and are able to rapidly divide and differentiate into effector cells. Stimulating memory cells to rapidly produce very high (effective) levels of persistent circulating antibodies is the basis for giving "booster"-type vaccinations to humans and pets. Thus, following the first exposure to an antigen the immune response (as evidenced by following the concentration of specific antibody in the serum) develops gradually over a period of days, reaches a low plateau within 2-3 weeks, and usually begins

to decline in a relatively short period of time. When the antigen is encountered a second time, a secondary (memory) response causes a rapid rise in the concentration of antibody, reaching a much higher level in the serum, which may persist for a relatively long period of time. A protective level of antibody may be reached by primary exposure alone, but usually to ensure a high level of protective antibody that persists over a long period of time, it is necessary to have repeated antigenic stimulation of the immune system.

An immunoglobulin molecule (abbreviated Ig), is a multimeric protein composed of two identical light chain polypeptides and two identical heavy chain polypeptides (H_2L_2) that are joined into a macromolecular complex by interchain disulfide bonds, *i.e.*, covalent bonds between the sulfhydryl groups of neighboring cysteine residues. There are various classes of human antibody proteins, each of which is produced by a specific clone of plasma cells. Five human immunoglobulin classes are defined on the basis of their heavy chain composition, and are named IgG, IgM, IgA, IgE, and IgD. The IgG-class and IgA-class antibodies are further divided into subclasses, namely, IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2. Intrachain disulfide bonds join different areas of the same polypeptide chain, which results in the formation of loops that, along with adjacent amino acids, constitute the immunoglobulin domains. At the amino-terminal portion (also called the "NH₂-terminus" or the "N-terminus"), each light chain and each heavy chain has a single variable region that shows considerable variation in amino acid composition from one antibody to another. The light chain variable region, V_L , associates with the variable region of a heavy chain, V_H , to form the antigen binding site of the immunoglobulin, called the Fv.

In addition to variable regions, each of the antibody chains has one or more constant regions. Light chains have a single constant region domain. Thus, light chains have one variable region and one constant region. Heavy chains have several constant region domains. The heavy chains in IgG, IgA, and IgD antibodies have three constant region domains, which are designated CH1, CH2, and CH3, and the heavy chains in IgM and IgE antibodies have four constant region domains, CH1, CH2, CH3 and CH4. Thus, heavy chains have one variable region and three or four constant regions. Immunoglobulin structure and function are reviewed, for example, in Harlow *et al.*, Eds., *Antibodies: A Laboratory Manual*, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988).

The heavy chains of immunoglobulins can also be divided into three functional regions: the Fd region (a fragment comprising V_H and CH1, *i.e.*, the two N-terminal domains of the heavy chain), the hinge region, and the Fc region (the "fragment crystallizable" region, derived from constant regions and formed after pepsin digestion).

5 The Fd region in combination with the light chain forms an Fab (the "fragment antigen-binding"). Because an antigen will react stereochemically with the antigen-binding region at the amino terminus of each Fab the IgG molecule is divalent, *i.e.*, it can bind to two antigen molecules. The Fc contains the domains that interact with immunoglobulin receptors on cells and with the initial elements of the complement cascade. Thus, the Fc
10 fragment is generally considered responsible for the effector functions of an immunoglobulin, such as complement fixation and binding to Fc receptors. Pepsin sometimes also cleaves before the third constant domain (CH3) of the heavy chain to give a large fragment F(abc) and a small fragment pFcb. These terms are also used for analogous regions of the other immunoglobulins. The hinge region, found in IgG, IgA, and
15 IgD class antibodies, acts as a flexible spacer, allowing the Fab portion to move freely in space. In contrast to the constant regions, the hinge domains are structurally diverse, varying in both sequence and length among immunoglobulin classes and subclasses.

For example, the length and flexibility of the hinge region varies among the IgG subclasses. The hinge region of IgG1 reportedly encompasses amino acids 216-231
20 and because it is freely flexible, the Fab fragments can rotate about their axes of symmetry and move within a sphere centered at the first of two inter-heavy chain disulfide bridges. IgG2 has a shorter hinge than IgG1, reportedly 12 amino acid residues and four disulfide bridges. The hinge region of IgG2 lacks a glycine residue, it is relatively short and contains a rigid poly-proline double helix, stabilised by extra inter-heavy chain disulfide bridges.
25 These properties restrict the flexibility of the IgG2 molecule. IgG3 differs from the other subclasses by its unique extended hinge region (about four times as long as the IgG1 hinge), and is reported to contain 62 amino acids (including 21 prolines and 11 cysteines), forming an inflexible poly-proline double helix. In IgG3 the Fab fragments are relatively far away from the Fc fragment, giving the molecule a greater flexibility. The elongated
30 hinge in IgG3 is also responsible for its higher molecular weight compared to the other subclasses. The hinge region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2. The flexibility of the hinge region reportedly

decreases in the order IgG3>IgG1>IgG4>IgG2. The four IgG subclasses also differ from each other with respect to their effector functions. This difference is related to differences in structure, including with respect to the interaction between the variable region, Fab fragments, and the constant Fc fragment. Nevertheless, aside from glycosylation within the CH2 region, for example, and in spite of this knowledge there are no set rules or conventions regarding means or methods to change features, including sequences, of these subclasses of molecule to change, control, add, or remove different functions, for example, ADCC, CDC, and other functions.

According to crystallographic studies, the immunoglobulin hinge region can be further subdivided functionally into three regions: the upper hinge region, the core region, and the lower hinge region. Shin *et al.*, 1992 *Immunological Reviews* 130:87. The upper hinge region includes amino acids from the carboxyl end of CH1 to the first residue in the hinge that restricts motion, generally the first cysteine residue that forms an interchain disulfide bond between the two heavy chains. The length of the upper hinge region correlates with the segmental flexibility of the antibody. The core hinge region contains the inter-heavy chain disulfide bridges, and the lower hinge region joins the amino terminal end of the CH2 domain and includes residues in CH2. *Id.* The core hinge region of human IgG1 contains the sequence Cys-Pro-Pro-Cys which, when dimerized by disulfide bond formation, results in a cyclic octapeptide believed to act as a pivot, thus conferring flexibility. The hinge region may also contain one or more glycosylation sites, which include a number of structurally distinct types of sites for carbohydrate attachment. For example, IgA1 contains five glycosylation sites within a 17 amino acid segment of the hinge region, conferring resistance of the hinge region polypeptide to intestinal proteases, considered an advantageous property for a secretory immunoglobulin.

Conformational changes permitted by the structure and flexibility of the immunoglobulin hinge region polypeptide sequence may also affect the effector functions of the Fc portion of the antibody. Three general categories of effector functions associated with the Fc region include (1) activation of the classical complement cascade, (2) interaction with effector cells, and (3) compartmentalization of immunoglobulins. The different human IgG subclasses vary in the relative efficacies with which they fix complement, or activate and amplify the steps of the complement cascade. *See, e.g.*, Kirschfink, 2001 *Immunol. Rev.* 180:177; Chakraborti *et al.*, 2000 *Cell Signal* 12:607;

Kohl *et al.*, 1999 *Mol. Immunol.* 36:893; Marsh *et al.*, 1999 *Curr. Opin. Nephrol. Hypertens.* 8:557; Speth *et al.*, 1999 *Wien Klin. Wochenschr.* 111:378.

Complement-dependent cytotoxicity (CDC) is believed to be a significant mechanism for clearance of specific target cells such as tumor cells. CDC is a stream of events that consists of a series of enzymes that become activated by each other in a cascade fashion. Complement has an important role in clearing antigen, accomplished by its four major functions: (1) local vasodilation; (2) attraction of immune cells, especially phagocytes (chemotaxis); (3) tagging of foreign organisms for phagocytosis (opsonization); and (4) destruction of invading organisms by the membrane attack complex (MAC attack). The central molecule is the C3 protein. It is an enzyme that is split into two fragments by components of either the classical pathway or the alternative pathway. Antibodies, especially IgG and IgM, induce the classical pathway while the alternative pathway is nonspecifically stimulated by bacterial products like lipopolysaccharide (LPS). Briefly, the products of the C3 split include a small peptide C3a that is chemotactic for phagocytic immune cells and results in local vasodilation by causing the release of C5a fragment from C5. The other part of C3, C3b coats antigens on the surface of foreign organisms and acts to opsonize the organism for destruction. C3b also reacts with other components of the complement system to form an MAC consisting of C5b, C6, C7, C8 and C9.

In general, IgG1 and IgG3 most effectively fix complement, IgG2 is less effective, and IgG4 does not activate complement. Complement activation is initiated by binding of C1q, a subunit of the first component C1 in the cascade, to an antigen-antibody complex. Even though the binding site for C1q is located in the CH2 domain of the antibody, the hinge region influences the ability of the antibody to activate the cascade. For example, recombinant immunoglobulins lacking a hinge region are reportedly unable to activate complement. Shin *et al.*, 1992. Without the flexibility conferred by the hinge region, the Fab portion of the antibody bound to the antigen may not be able to adopt the conformation required to permit C1q to bind to CH2. *See id.* Hinge length and segmental flexibility have been reported to correlate with complement activation; however, the correlation is not absolute. Human IgG3 molecules with altered hinge regions that are as rigid as IgG4, for example, can still effectively activate the cascade.

The absence of a hinge region, or a lack of a functional hinge region, can also affect the ability of certain human IgG immunoglobulins to bind Fc receptors on immune effector cells. Binding of an immunoglobulin to an Fc receptor facilitates antibody-dependent cell-mediated cytotoxicity, which as noted above is presumed to be an important mechanism for the elimination of tumor cells. The human IgG Fc receptor (FcR) family is divided into three groups, FcγRI (CD64), which is capable of binding IgG with high affinity, and FcγRII (CD32) and FcγRIII (CD16), both of which are lower affinity receptors. The molecular interaction between each of the three receptors and an immunoglobulin has not been defined precisely, but experimental evidence indicates that residues in the hinge proximal region of the CH2 domain may be important to the specificity of the interaction between the antibody and the Fc receptor. IgG1 myeloma proteins and recombinant IgG3 chimeric antibodies that lack a hinge region are reportedly unable to bind FcγRI, perhaps because accessibility to CH2 is decreased. Shin *et al.*, 1993 *Intern. Rev. Immunol.* 10:177, 178-79.

Unusual and apparently evolutionarily unrelated exceptions to the H₂L₂ structure of conventional antibodies occur in some isotypes of the immunoglobulins found in camelids (camels, dromedaries and llamas; Hamers-Casterman *et al.*, 1993 *Nature* 363:446; Nguyen *et al.*, 1998 *J. Mol. Biol.* 275:413), nurse sharks (Roux *et al.*, 1998 *Proc. Nat. Acad. Sci. USA* 95:11804), and in the spotted ratfish (Nguyen, *et al.*, "Heavy-chain antibodies in Camelidae; a case of evolutionary innovation," 2002 *Immunogenetics* 54(1):39-47). These antibodies can apparently form antigen-binding regions using only heavy chain variable region, *i.e.*, these functional antibodies are homodimers of heavy chains only (referred to as "heavy-chain antibodies" or "HCAbs"). In both species, these variable regions often contain an extended third complementarity determining region (CDR3) that may help compensate for the lack of a light chain variable region, and there are frequent disulfide bonds between CDR regions that presumably help to stabilize the binding site. Muyldermans *et al.*, 1994 *Prot. Engineer.* 7:1129; Roux *et al.*, 1998. However, the precise function of the heavy chain-only "antibodies" is unknown, and the evolutionary pressure leading to their formation has not been identified. See, *e.g.*, Nguyen, *et al.*, 2002, *supra*. Camelids, including camels, llamas, and alpacas, also express conventional H₂L₂ antibodies, and the heavy chain-only antibodies thus do not appear to be present in these animals simply as an alternative antibody structure.

Variable regions (V_{HH}) of the camelid heavy chain-only immunoglobulins and conventional (H_2L_2) heavy chain variable regions contain amino acid differences, including differences at several positions that may be involved in the interface between conventional V_{H} and V_{L} domains. Nguyen *et al.*, 1998 *J. Mol. Biol* **275**:413; Muyldermans *et al.*, 1994 *Prot. Engineer.* **7**:1129. Camelid V_{HH} reportedly recombines with IgG2 and IgG3 constant regions that contain hinge, CH2, and CH3 domains and lack a CH1 domain. Hamers-Casterman *et al.*, 1993 *Nature* **363**:446. Interestingly, V_{HH} are encoded by a chromosomal locus distinct from the V_{H} locus (Nguyen *et al.*, 1998, *supra*), indicating that camelid B cells have evolved complex mechanisms of antigen recognition and differentiation. Thus, for example, llama IgG1 is a conventional (H_2L_2) antibody isotype in which V_{H} recombines with a constant region that contains hinge, CH1, CH2 and CH3 domains, whereas the llama IgG2 and IgG3 are heavy chain-only isotypes that lack CH1 domains and that contain no light chains.

The classes of immunoglobulins have different physical and chemical characteristics and they exhibit unique biological properties. Their synthesis occurs at different stages and rates during an immune response and/or during the course of an infection. Their importance and functions in host resistance (immunity) are different.

Immunoglobulin G (IgG), a protein with a molecular weight of about 150,000 daltons (150kD), is the predominant Ig in the serum. It makes up about 80% of the total antibody found in an animal at any given time, being 75% of the total serum antibody. It can diffuse out of the blood stream into the extravascular spaces and it is the most common Ig found there. Its concentration in tissue fluids is increased during inflammation, and it is particularly effective at the neutralization of bacterial extracellular toxins and viruses. It also has opsonizing ability and complement-fixing ability. The polypeptide composition of the Fc region of all IgG1 antibody molecules is relatively constant regardless of antibody specificity; however, as noted above, the Fab regions always differ in their exact amino acid sequences depending upon their antigenic specificity. Specific amino acid regions of the Fc portion of the molecule are recognized by receptors on phagocytes and certain other cells, and the Fc domain contains a peptide region that will bind to and activate complement, which is often required for the manifestation of antibody-mediated immunity. Because the IgG molecule is divalent, it can cross-link antigen molecules, which may lead to precipitation or agglutination of

antigens; if IgG is bound to antigen on a microbial cell or surface, its Fc region may provide an extrinsic ligand that will be recognized by specific receptors on phagocytes. Microbial cells or viruses coated with IgG molecules are opsonized for phagocytosis, and opsonized pathogens are taken up and destroyed much more readily by phagocytes than their non-opsonized counterparts. IgG, as well as IgM and IgA, will neutralize the activity of toxins, including bacterial exotoxins. Furthermore, cross-linked IgG molecules on the surface of a cell can bind and activate complement from the serum and set off a cascade of reactions that can lead to destruction of the cell.

IgM is the first immunoglobulin to appear in the blood stream during the course of an infection. It is mainly confined to the bloodstream and provides protection against blood-borne pathogens. IgM makes up about 10% serum immunoglobulins, and is arranged to resemble a pentamer of five immunoglobulin molecules (having a molecular weight of about 900kD) tethered together at by their Fc domains. In addition to covalent linkages between the monomeric subunits, the pentamer is stabilized by a 15kd polypeptide called J chain. IgM, therefore, has a theoretical "valence" of ten (*i.e.*, it has ten exposed Fab domains). Probably, the most important role of IgM is its ability to function early in the immune responses against blood-borne pathogens given its efficiency in agglutinating particulate antigens. IgM binds also complement strongly and IgM antibodies bound to a microbial surface act as opsonins, rendering the microbe more susceptible to phagocytosis. In the presence of complement and IgM whole microbial cells may be killed and lysed. As noted above, IgM also appears on the surfaces of mature B cells as a transmembranous monomer where it functions as an antigen receptor, capable of activating B cells when bound to antigen.

Gene rearrangement at the immunoglobulin loci during lymphoid development generates a repertoire of B lymphocytes that express a diversity of antigen receptors. The gene rearrangement, which is catalysed by the rearrangement-activating gene ("RAG") recombinase, integrates the immunoglobulin V, D and J gene segments to yield productively rearranged immunoglobulin genes that encode the heavy and light chains of IgM antibodies. The diversity of IgM antibodies in this primary repertoire is achieved through combinatorial mechanisms (the choice of V, D and J gene segments utilized in a particular antibody), as well as junctional diversity. The joining of V, D and J gene segments is somewhat imprecise, and nucleotides may be inserted at the junction in a

non-templated manner. There is therefore a very high degree of resultant diversity at the V-D-J borders. This contributes in a major way to the structural diversity of the third complementarity determining region of the antibody, a region that often plays a critical role in antigen recognition. This primary repertoire of IgM antibodies comprises a few million different structures. The size of this repertoire means that any incoming antigen is likely to encounter an antibody that recognizes it with acceptable affinity. A high-affinity binding site is unlikely to be available for most incoming antigens (the repertoire is not large enough), but the affinity of the available IgM antibodies in the primary repertoire will vary from antigen to antigen. If an epitope is re-iterated at high density on the surface of the antigen (*e.g.*, a repeated structure on the surface of a virus or bacterium), then an IgM antibody may nevertheless be effective in mediating clearance of the organism, despite the low affinity of the individual interaction between antigenic epitope and immunoglobulin combining site. The density of the epitopes may allow multivalent interactions with IgM, leading to a high-avidity interaction, providing that a suitable spacing of antigenic epitopes can occur. Nevertheless, to ensure an effective and specific response, especially when the concentration of antigen is low (as may occur when the body is faced with a very small number of infecting viral particles), it would be preferable if high-affinity antibodies were available for neutralizing, for example, an infecting organism. The size of the primary repertoire militates against the likelihood of such high-affinity antibodies being present in this repertoire. The immune system therefore operates using a two-stage strategy. The primary repertoire of IgM antibodies is generated by a process of gene rearrangement and takes place prior to antigen encounter during early lymphocyte development. However, once foreign antigen has been encountered, those B cells in the primary repertoire that encode suitable (albeit low-affinity) antibodies are selectively expanded and subjected to an iterative alternation of directed hypermutation and antigen-mediated selection. This allows a significant maturation in affinity of the antigen-specific antibodies that are produced. Antigen triggering also drives isotype switch recombination. Thus, in the absence of external antigen stimulation and any maternally derived immunoglobulin, the serum will only contain a diversity of unmutated IgM molecules that have been generated by gene rearrangement. This repertoire shifts with age as a result of continuous antigen exposure, such that the majority of the serum immunoglobulin in older animals is

composed of mutated IgG (and IgA) molecules whose specificities have developed as a consequence of antigen selection.

IgA exists as a H₂L₂ monomer of about 160kD in serum and, in secretions, as a dimer of the H₂L₂ monomer of about 400kD. As with IgM, polymerization (dimerization) is via a J-chain. IgA has two subclasses based on different heavy chains, IgA1 and IgA2. IgA1 is produced in bone marrow and makes up most of the serum IgA. Both IgA1 and IgA2 are synthesized in GALT (gut associated lymphoid tissues) to be secreted onto the mucosal surfaces. Because IgA may be synthesized locally and secreted in the seromucous secretions of the body, it is sometimes referred to as secretory antibody or sIgA. Quantitatively, IgA is synthesized in amounts greater than IgG, but it has a short half life in serum (6 days), and it is lost in secretory products. The concentration of IgA in serum is about 15% of the total antibody. Secretion of dimeric IgA is mediated by a 100kD glycoprotein called secretory component. It is the addition of the secretory piece to IgA molecules that accounts for their ability to exit the body to mucosal surfaces via the exocrine glands. IgM can be transported similarly and makes up a small proportion of secretory antibodies. Secretory IgA is the predominant immunoglobulin present in gastrointestinal fluids, nasal secretions, saliva, tears and other mucous secretions of the body. IgA antibodies are important in resistance to infection of the mucosal surfaces of the body, particularly the respiratory, intestinal and urogenital tracts. IgA acts as a protective coating for the mucous surfaces against microbial adherence or initial colonization. It can also neutralize toxin activity on mucosal surfaces. Fc receptors for IgA-coated microorganisms found on monocytes and neutrophils derived from the respiratory mucosa suggest that IgA may have a role in the lung, at least, in opsonization of pathogens. Secretory IgA is also transferred via the milk, *i.e.*, the colostrum, from a nursing mother to a newborn, which provides passive immunity to many pathogens, especially those that enter by way of the GI tract.

IgE is an immunoglobulin of about 190kD that accounts for about 0.002% of the total serum immunoglobulins. It is produced by plasma cells below the respiratory and intestinal epithelia. The majority of IgE is bound to tissue cells, especially mast cells. If an infectious agent succeeds in penetrating the IgA barrier, it comes up against the next line of defense, the MALT (mucosa-associated lymphoid tissues) system that is managed by IgE. IgE is bound very firmly to specific IgE Fc-receptors on mast cells. Contact with

antigen leads to release of mediators of inflammation from the mast cells, which effectively recruits various agents of the immune response including complement, chemotactic factors for phagocytes, T cells, *etc.* Although a well-known manifestation of this reaction is a type of immediate hypersensitivity reaction called atopic allergy (*e.g.*, hives, asthma, hay fever, *etc.*), the MALT responses act as a defense mechanism because they amplify the inflammatory response and may facilitate rejection of a pathogen.

IgD is a molecule of about 175kd that resembles IgG in its monomeric form. IgD antibodies are found for the most part on the surfaces of B lymphocytes. The same cells may also carry IgM antibody. As noted above, it is thought that IgD and IgM function as mutually interacting antigen receptors for control of B cell activation and suppression. Hence, IgD may have an immunoregulatory function.

In addition to opsonization, activation of complement, and ADCC, antibodies have other functions in host defense including steric hindrance, toxin neutralization, agglutination, and precipitation. With regard to steric hindrance, it is understood that antibodies combine with the surfaces of microorganisms and may block or prevent their attachment to susceptible cells or mucosal surfaces. Antibody against a viral component can block attachment of the virus to susceptible host cells and thereby reduce infectivity. Secretory IgA can block attachment of pathogens to mucosal surfaces. Toxin-neutralizing antibodies (antitoxins) can also react with a soluble bacterial toxin and block the interaction of the toxin with its specific target cell or substrate. Antibodies can also combine with the surfaces of microorganisms or soluble antigens and cause them to agglutinate or precipitate. This reduces the number of separate infectious units and makes them more readily phagocytosed because the clump of particles is larger in size. Phagocytes may remove floccules or aggregates of neutralized toxin.

Antibodies have been proposed for use in therapy. Animals, including humans and mice have the ability to make antibodies able to recognize (by binding to) virtually any antigenic determinant and to discriminate between similar epitopes. Not only does this provide the basis for protection against disease organisms, but it also makes antibodies attractive candidates to target other types of molecules found in the body, such as receptors or other proteins present on the surface of normal cells and molecules present uniquely on the surface of cancer cells. Thus the remarkable specificity of antibodies makes them promising agents for human therapy.

Initial antibody preparations available for use, such as intravenous gammaglobulins, included animal and human antisera that were used *in vivo* to destroy bacteria (tetanus, pneumococcus) and neutralize virus (hepatitis A and B, rabies, cytomegalovirus, and varicella zoster) in the blood of infected individuals. Possibly the most important early application was the use of endotoxins. However, there are problems associated with the use of antibodies in human therapy because the response of the immune system to any antigen, even the simplest, is "polyclonal," *i.e.*, the system manufactures antibodies of a great range of structures both in their binding regions as well as in their effector regions. Polyclonal antibody treatment was also associated with unwanted side effects. In addition to the polyclonal nature of these antibody preparations, there was the risk of infection from contaminating viruses. Serum sickness and anaphylaxis were also considered limiting factors. Furthermore, even if one were to isolate a single antibody-secreting cell, and place it in culture, it would die out after a few generations because of the limited growth potential of all normal somatic cells.

Until the late 1970s, polyclonal antibodies obtained from the blood serum of immunized animals provided the only source of antibodies for research or treatment of disease. Isolation of specific antibodies was essentially impossible until Kohler and Milstein discovered how to make "monoclonal antibodies" that would have a single specificity, that would all be alike due to manufacture by a single clone of plasma cells and that could be grown indefinitely. This technique was described in a 1975 publication (*Nature* 256:495-97), and Köhler and Milstein received the 1984 Nobel Prize in Medicine for their work.

The first step in Kohler and Milstein's technique for production of monoclonal antibodies involves immunizing an experimental animal with the antigen of interest. In most of their experiments, Kohler and Milstein injected a mouse with sheep red blood cells. The mouse's body initiates an immune response and begins producing antibodies specific to the antigen. The mouse's spleen is then removed and B cells producing the antibody of interest are isolated. Tumor-producing cells that have been grown in culture are then fused with the B lymphocytes using polyethylene glycol in order to produce a "hybridoma." Only hybridomas resulting from the fusion will survive. The spleen lymphocyte has a limited life span, so any B cells that did not fuse with a myeloma will die in the culture. Additionally, those cells that lack the antibody-producing aspect of

the B cell will not secrete the enzyme HGPRT, which is required for growth in the hypoxanthine-aminopterin-thymidine (HAT) medium. The HAT medium, on which the cells are grown, inhibits the pathway for nucleotide synthesis. Cells that produce HGPRT can bypass this pathway and continue to grow. By placing the fused cells in a HAT medium, true hybridomas can be isolated. The isolated hybridoma cells are then screened for specificity to the desired antigen. Because each hybridoma descends from one B cell, it makes copies of only one antibody. The hybridoma that produces the antibody of interest is grown in culture to produce large amounts of monoclonal antibodies, which are then isolated for further use. The technique is called somatic cell hybridization, and the resulting hybridoma (selected for both immortality and production of the specific antibody of interest) may be cultured indefinitely, *i.e.*, it is a potentially immortal cell line.

Monoclonal antibodies are now widely used as diagnostic and research reagents. However, their introduction into human therapy has been much slower. One principal difficulty is that mouse antibodies are "seen" by the human immune system as foreign. The human patient mounts an immune response against them, producing HAMA ("human anti-mouse antibodies"). These not only cause the therapeutic antibodies to be quickly eliminated from the host, but also form immune complexes that cause damage to the kidneys.

Two approaches have been used in an attempt to reduce the problem of HAMA. The first is the production of chimeric antibodies in which the antigen-binding part (variable regions) of a mouse monoclonal antibody is fused to the effector part (constant region) of a human antibody using genetic engineering. In a second approach, rodent antibodies have been altered through a technique known as complementarity determining region (CDR) grafting or "humanization." In this process, the antigen binding sites, which are formed by three CDRs of the heavy chain and three CDRs of the light chain, are excised from cells secreting rodent mAb and grafted into the DNA coding for the framework of the human antibody. Because only the antigen-binding site CDRs, rather than the entire variable domain of the rodent antibody are transplanted, the resulting humanized antibody (a second generation or "hyperchimeric" antibody) is reportedly less immunogenic than a first generation chimeric antibody. This process has been further improved to include changes referred to as "reshaping" (Verhoeyen, *et al.*, "Reshaping human antibodies: grafting an anti-lysozyme activity," 1988 *Science* 239:1534-1536;

Riechmann, *et al.*, "Reshaping human antibodies for therapy," 1988 *Nature* 332:323-337; Tempest, *et al.*, "Reshaping human monoclonal antibody to inhibit respiratory syncytial virus infection in vivo," *Bio/Technol* 1991 9:266-271), "hyperchimerization" (Queen, *et al.*, "A humanized antibody that binds to the human interleukin 2 receptor," 1989 *Proc Natl Acad Sci USA* 86:10029-10033; Co, *et al.*, "Humanized antibodies for antiviral therapy," 1991 *Proc Natl Acad Sci USA* 88:2869 -2873; Co, *et al.*, "Chimeric and humanized antibodies with specificity for the CD33 antigen," 1992 *J Immunol* 148:1149-1154), and "veneering" (Mark, *et al.*, "Derivation of therapeutically active humanized and veneered anti-CD18 antibodies. In: Metcalf BW, Dalton BJ, eds. Cellular adhesion: molecular definition to therapeutic potential. New York: Plenum Press, 1994:291-312).

In the reshaping process on the basis of homology, the rodent variable region is compared with the consensus sequence of the protein sequence subgroup to which it belongs. Similarly, the selected human constant region accepting framework is compared with its family consensus sequence. Gussowal, *et al.*, "Humanization of monoclonal antibodies," 1991 *Meth Enzymol* 203:99-121. The sequence analyses identify residues, which may have undergone mutation during the affinity maturation procedure and may therefore be idiosyncratic. Inclusion of the more common human residues is said to lessen immunogenicity problems by replacing human acceptor idiosyncratic residues. Further, the reshaping process is said to allow comparison of human and rodent consensus sequences to identify any systematic "species" differences. RSV19 antibodies were humanized by employing this procedure. Taylor *et al.*, "Humanized monoclonal antibody to respiratory syncytial virus," 1991 *Lancet* 337:1411-1412; Tempest, *et al.*, "Reshaping a human monoclonal antibody to inhibit human respiratory syncytial virus infection in vivo," 1991 *Bio/Technol* 9:266-271.

Hyperchimerization is an alternative method of identifying residues outside CDR regions that are likely to be involved in the reconstitution of binding activity. In this method, the human sequences are compared with murine variable region sequences and the one with highest homology is selected as the acceptor framework. As in the reshaping procedure, the "idiosyncratic" residues are replaced by more commonly occurring human residues. The non-CDR residues that may be interacting with the CDR sequences are identified. Finally, it is determined which one of these residues is to be included in the variable region framework. Humanized antibodies against CD33 antigen were reportedly

developed by this method. Co, *et al.*, "Chimeric and humanized antibodies with specificity for the CD33 antigen," 1992 *J Immunol* 148:1149-154. See also Carter, *et al.*, "Humanization of an anti-p185 HER2 antibody for human cancer therapy," 1992 *Proc Natl Acad Sci USA* 89:4285-4289.

5 The displayed surface of the protein is the primary determinant of its immunogenicity. A humanized murine antibody can thus be made less immunogenic by replacing exposed residues that differ from those commonly found in human antibodies. This method of humanization is referred to as "veneering." Appropriate replacement of the outer residues may have little or no impact on the inner domains or interdomain
10 framework. Veneering is a two-step process. In the first step, the most homologous human variable regions are selected and compared by each single residue to the corresponding mouse variable regions. In the second step, the residues present in the human homologue replace the mouse framework residues, which differ from its human homologue. This replacement involves only those residues that are on the surface and at
15 least partially exposed.

 Nevertheless, it took more than a quarter century of research for monoclonal antibody technology and genetic engineering methods to result in the development of immunoglobulin molecules for treatment of human diseases. Indeed, it was not until the
past five years that monoclonal antibodies became an expanding class of therapeutics. See
20 Glennie MJ and van de Winkel JG, *Drug Discov Today* 2003 Jun 1;8(11):503-10; Souriau C and Hudson PJ, "Recombinant antibodies for cancer diagnosis and therapy," 2003 *Expert Opin Biol Ther.* 3(2):305-18. See also Pendley C, *et al.*, "Immunogenicity of therapeutic monoclonal antibodies," 2003 *Curr Opin Mol Ther.* 5(2):172-9.

 All the same, an average of less than one therapeutic antibody per year has
25 been introduced to the market beginning in 1986, eleven years after the publication of monoclonal antibodies. Five murine monoclonal antibodies were introduced into human medicine over a ten year period from 1986-1995, including "muromonab-CD3" (OrthoClone OKT3®), which binds to a molecule on the surface of T cells and was launched in 1986 to prevent acute rejection of organ transplants; "edrecolomab"
30 (Panorex®), launched in 1995 for treatment of colorectal cancer; "odulimomab" (Antilfa®), launched in 1997 for use in transplant rejection; and, "ibritumomab" (Zevalin®) (yixetan), launched in 2002 for use in non-Hodgkin's lymphoma. Additionally, one

monoclonal Fab, "abciximab" (ReoPro®), was launched in 1995. It inhibits the clumping of platelets by binding the receptors on their surface that normally are linked by fibrinogen and may be helpful in preventing reclogging of the coronary arteries in patients who have undergone angioplasty. Three chimeric monoclonal antibodies were also launched:

- 5 "rituximab" (Rituxan®), in 1997, which binds to the CD20 molecule found on most B cells and is used to treat B cell lymphomas; "basiliximab" (Simulect®), in 1998 for transplant rejection; and "infliximab" (Remicade®) which binds to tumor necrosis factor-alpha (TNF- α), in 1998 for treatment of rheumatoid arthritis and Crohn's disease. Additionally, "abciximab" (ReoPro®), a 47.6 kD Fab fragment of the chimeric human-murine
- 10 monoclonal antibody 7E3 that binds to the glycoprotein (GP) IIb/IIIa receptor of human platelets, was launched in 1995 as an adjunct to percutaneous coronary intervention for the prevention of cardiac ischemic complications in patients undergoing percutaneous coronary intervention. Finally, seven "humanized" monoclonals were launched from 1997-2003: "daclizumab" (Zenapax®) in 1997, which binds to part of the IL-2 receptor
- 15 produced at the surface of activated T cells and is used to prevent acute rejection of transplanted kidneys; "palivizumab" (Synagis®) in 1998 for RSV; "trastuzumab" (Herceptin®) in 1998, which binds HER-2, a growth factor receptor found on breast cancers cells; "gemtuzumab" (Mylotarg®) in 2000, which is a conjugate of a monoclonal antibody that binds CD33, a cell-surface molecule expressed by the cancerous cells in
- 20 acute myelogenous leukemia (AML) but not found on the normal stem cells needed to repopulate the bone marrow; and "alemtuzumab" (MabCampath®) in 2001, which binds to CD52, a molecule found on white blood cells and has produced temporary remission of chronic lymphocytic leukemia; "adalimumab" (Humira® (D2E7)), a human anti-TNF monoclonal containing human-derived heavy chain and light chain variable regions and
- 25 human IgG: κ constant regions was launched in 2002 for the treatment of rheumatoid arthritis; and, "omalizumab" (Xolair®), which binds to IgE and prevents it from binding to mast cells was approved in 2003 for the treatment of adults and adolescents over 12 years of age with moderate to severe persistent asthma who have a positive skin test or *in vitro* reactivity to a perennial aeroallergen and whose symptoms are inadequately controlled
- 30 with inhaled corticosteroids.

Thus, protein engineering has been applied in an effort to diminish problems related to immunogenicity of administered recombinant immunoglobulin polypeptides and

to try to alter antibody effector functions. However, problems remain. For example, the majority of cancer patients treated with rituximab relapse, generally within about 6-12 months, and fatal infusion reactions within 24 hours of rituximab infusion have been reported. These fatal reactions followed an infusion reaction complex that included
5 hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation or cardiogenic shock. Acute renal failure requiring dialysis with instances of fatal outcome has also been reported in the setting of tumor lysis syndrome following treatment with rituximab, as have severe mucocutaneous reactions, some with fatal outcome. Additionally, high doses of rituximab are required for intravenous injection
10 because the molecule is large, approximately 150 kDa, and diffusion is limited into the lymphoid tissues where many tumor cells reside.

Trastuzumab administration can result in the development of ventricular dysfunction and congestive heart failure, and the incidence and severity of cardiac dysfunction has been reported to be particularly high in patients who received trastuzumab
15 in combination with anthracyclines and cyclophosphamide. Trastuzumab administration can also result in severe hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary events.

Patients receiving daclizumab immunosuppressive therapy are at increased risk for developing lymphoproliferative disorders and opportunistic infections, and it is not
20 known whether daclizumab use will have a long-term effect on the ability of the immune system to respond to antigens first encountered during daclizumab-induced immunosuppression.

Hepatotoxicity, including severe hepatic veno-occlusive disease (VOD), has also been reported in association with the use of gemtuzumab as a single agent, as part of a
25 combination chemotherapy regimen, and in patients without a history of liver disease or hematopoietic stem-cell transplant (HSCT). Patients who receive gemtuzumab either before or after HSCT, patients with underlying hepatic disease or abnormal liver function, and patients receiving gemtuzumab in combinations with other chemotherapy may be at increased risk for developing severe VOD. Death from liver failure and from VOD has
30 been reported in patients who received gemtuzumab, and it has been cautioned that even careful monitoring may not identify all patients at risk or prevent the complications of hepatotoxicity.

Hepatotoxicity was also reported in patients receiving alemtuzumab. Serious and, in some rare instances fatal, pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia have occurred in patients receiving alemtuzumab therapy. Alemtuzumab can also result in serious infusion reactions as well as opportunistic infections.

In patients treated with adalimumab, serious infections and sepsis, including fatalities, have been reported, as has the exacerbation of clinical symptoms and/or radiographic evidence of demyelinating disease, and patients treated with adalimumab in clinical trials had a higher incidence of lymphoma than the expected rate in the general population.

Serious adverse reactions in clinical studies with omalizumab have included malignancies and anaphylaxis, in which the observed incidence of malignancy among omalizumab-treated patients (0.5%) was numerically higher than among patients in control groups (0.2%).

Smaller immunoglobulin molecules have been constructed in an effort to overcome various problems associated with whole immunoglobulin therapy. Single chain immunoglobulin variable region fragment polypeptides (scFvs) are made of an immunoglobulin heavy chain variable domain joined via a short linker peptide to an immunoglobulin light chain variable domain. Huston *et al.*, 1988 *Proc. Natl. Acad. Sci. USA*, **85**:5879-83. It has been suggested that the smaller size of scFv molecules may lead to more rapid clearance from plasma and more effective penetration into tissues than whole immunoglobulins. See, e.g., Jain, 1990 *Cancer Res.* **50**:814s-819s. An anti-tumor scFv was reported to show more rapid tumor penetration and more even distribution through the tumor mass than the corresponding chimeric antibody. Yokota *et al.*, *Cancer Res.* **52**:3402-08 (1992).

Despite advantages that scFv molecules may have with regard to serotherapy, drawbacks to this therapeutic approach also exist. For example, rapid clearance of scFv may prevent delivery of a minimum effective dose to the target tissue. Additionally, manufacturing adequate amounts of scFv for administration to patients has been challenging due to difficulties in expression and isolation of scFv that adversely affect yields. During expression, scFv molecules lack stability and often aggregate due to pairing of variable regions from different molecules. Furthermore, production levels of scFv

molecules in mammalian expression systems are reportedly low, which may limit the potential for efficient manufacturing of scFv molecules for therapy. Davis *et al.*, 1990 *J. Biol. Chem.* 265:10410-18; Traunecker *et al.*, 1991 *EMBO J.* 10:3655-59. Strategies for means to improve production have been explored, and reportedly include the addition of glycosylation sites to variable regions. See, e.g., U.S. Patent No. 5,888,773; Jost *et al.*, 1994 *J. Biol. Chem.* 269:26267-73. Another disadvantage to the use of scFvs for therapy is the lack of effector function. An scFv that lacks the cytolytic functions, ADCC, and complement dependent-cytotoxicity may be less effective or ineffective for treating disease. Even though development of scFv technology began over 12 years ago, there are currently no scFv products approved for therapy.

Alternatively, it has been proposed that fusion of an scFv to another molecule, such as a toxin, could take advantage of the specific antigen-binding activity and the small size of an scFv to deliver the toxin to a target tissue. Chaudary *et al.*, 1989 *Nature* 339:394; Batra *et al.*, 1991 *Mol. Cell. Biol.* 11:2200. Conjugation or fusion of toxins to scFvs has thus been offered as an alternative strategy to provide potent, antigen-specific molecules, but dosing with such conjugates or chimeras can be limited by excessive and/or non-specific toxicity due to the toxin moiety of such preparations. Toxic effects may include supraphysiological elevation of liver enzymes and vascular leak syndrome, and other undesired effects. In addition, immunotoxins are themselves highly immunogenic upon administration to a host, and host antibodies generated against the immunotoxin limit potential usefulness for repeated therapeutic treatments of an individual.

Fusion proteins in which immunoglobulin constant region polypeptide sequences are present and nonimmunoglobulin sequences are substituted for the antibody variable regions have also been investigated. For example, CD4, the T cell surface protein recognized by HIV, was recombinantly fused to an immunoglobulin Fc effector domain, and an IL-2-IgG1 fusion protein reportedly effected complement-mediated lysis of IL-2 receptor-bearing cells. See Sensel *et al.*, *Chem. Immunol.* 65:129-158 (1997). An extensive introduction as well as detailed information about all aspects of recombinant antibody technology can be found in the textbook "Recombinant Antibodies" (John Wiley & Sons, NY, 1999). A comprehensive collection of detailed antibody engineering lab

Protocols can be found in R. Kontermann and S. Dübel (eds.), "The Antibody Engineering Lab Manual" (Springer Verlag, Heidelberg/New York, 2000).

Diseases and disorders thought to be amenable to some type of immunoglobulin therapy include cancer and immune system disorders. Cancer includes a broad range of diseases, affecting approximately one in four individuals worldwide. Rapid and unregulated proliferation of malignant cells is a hallmark of many types of cancer, including hematological malignancies. Although patients with a hematologic malignant condition have benefited from advances in cancer therapy in the past two decades, Multani *et al.*, 1998 *J. Clin. Oncology* 16:3691-3710, and remission times have increased, most patients still relapse and succumb to their disease. Barriers to cure with cytotoxic drugs include, for example, tumor cell resistance and the high toxicity of chemotherapy, which prevents optimal dosing in many patients.

Nevertheless, patients have been treated with immunotherapeutics that target malignant cells, *i.e.*, to antigens expressed on tumor cells. With regard to the selection of tumor cell surface antigens suitable for use as immunotherapy targets, it is preferable that such a target antigen is not expressed by normal tissues, particularly where the preservation of such tissue is important to host survival. In the case of hematologic malignancy, malignant cells express many antigens that are not expressed on the surfaces of stem cells or other essential cells. Treatment of a hematologic malignant condition using a therapeutic regimen that depletes both normal and malignant cells of hematological origin has been acceptable where regeneration of normal cells from progenitors can occur after therapy has ended. Additionally, the target antigen is desirably expressed on all or virtually all clonogenic populations of tumor cells, and it is best that expression persists despite selective pressure from immunoglobulin therapy. Strategies that employ selection of a cell surface idiotype (*e.g.*, a particular idiotope) as a target for therapy of B cell malignancy have been limited by the outgrowth of tumor cell variants with altered surface idiotype expression, even where the antigen exhibits a high degree of tumor selectivity. Meeker *et al.*, 1985 *N. Engl. J. Med.* 312:1658-65. The selected antigen should also traffic properly after an immunoglobulin binds to it. Shedding or internalization of a cell surface target antigen after an immunoglobulin binds to the antigen may allow tumor cells to escape destruction, thus limiting the effectiveness of serotherapy. Finally, binding of an immunoglobulin to cell surface target antigens that transmit or transduce cellular activation

signals may result in improved functional responses to immunotherapy in tumor cells, and can lead to growth arrest and/or apoptosis. While all of these properties are important, the triggering of apoptosis after an immunoglobulin binds to the target antigen may also be a critical factor in achieving successful serotherapy.

5 Antigens that have been tested as targets for serotherapy of B and T cell malignancies include Ig idiotype (Brown *et al.*, 1989 *Blood* 73:651-61), CD19 (Hekman *et al.*, 1991 *Cancer Immunol. Immunother.* 32:364-72), Vlasveld *et al.*, 1995 *Cancer Immunol. Immunother.* 40:37-47), CD20 (Press *et al.*, 1987 *Blood* 69: 584-91), Maloney *et al.*, 1997 *J. Clin. Oncol.* 15:3266-74), CD21 (Scheinberg *et al.*, 1990 *J. Clin. Oncol.* 8:792-803), CD5 (Dillman *et al.*, 1986 *J. Biol. Respn. Mod.* 5:394-410), and CD52 (CAMPATH) (Pawson *et al.*, 1997 *J. Clin. Oncol.* 15:2667-72). Of these, greater benefit for therapy of B cell lymphomas has been obtained using molecules that target CD20. Other targets have been limited by biological properties of the antigen. For example, surface idiotype can be altered through somatic mutation, allowing tumor cell escape. CD5, CD21, and CD19 are
10 rapidly internalized after monoclonal antibody binding, allowing tumor cells to escape destruction unless monoclonal antibodies are conjugated with toxin molecules. CD22 is expressed on only a subset of B cell lymphomas, thereby limiting its usefulness, while CD52 is expressed on both T cells and B cells and may therefore generate
15 counterproductive immunosuppression by depletion.

20 Treatment of patients with low grade or follicular B cell lymphoma using a chimeric CD20 monoclonal antibody has been reported to induce partial or complete responses in patients. McLaughlin *et al.*, 1996 *Blood* 88:90a (abstract, suppl. 1); Maloney *et al.*, 1997 *Blood* 90:2188-95. However, as noted above, tumor relapse commonly occurs within six months to one year. Further improvements in serotherapy are needed to induce
25 more durable responses, for example, in low grade B cell lymphoma, and to allow effective treatment of high-grade lymphoma and other B cell diseases.

 Another approach has been to target radioisotopes to B cell lymphomas using monoclonal antibodies specific for CD20. While the effectiveness of therapy is reportedly increased, associated toxicity from the long *in vivo* half-life of the radioactive
30 antibody increases also, sometimes requiring that the patient undergo stem cell rescue. Press *et al.*, 1993 *N. Eng. J. Med.* 329:1219-1224; Kaminski *et al.*, 1993 *N. Eng. J. Med.* 329:459-65. Monoclonal antibodies to CD20 have also been cleaved with proteases to

yield F(ab')₂ or Fab fragments prior to attachment of radioisotope. This has been reported to improve penetration of the radioisotope conjugate into the tumor and to shorten the *in vivo* half-life, thus reducing the toxicity to normal tissues. However, these molecules lack effector functions, including complement fixation and/or ADCC.

5 CD20 was the first human B cell lineage-specific surface molecule identified by a monoclonal antibody. It is a non-glycosylated, hydrophobic 35 kDa B cell transmembrane phosphoprotein that has both amino and carboxy ends situated in the cytoplasm. Einfeld *et al.*, 1988 *EMBO J.* 7:711-17. CD20 is expressed by all normal mature B cells, but is not expressed by precursor B cells. Natural ligands for CD20 have
10 not been identified, and the function of CD20 in B cell biology is still incompletely understood.

Anti-CD20 monoclonal antibodies affect the viability and growth and growth of B cells. Clark *et al.*, 1986 *Proc. Natl. Acad. Sci. USA* 83:4494-98. Extensive cross-linking of CD20 can induce apoptosis in B lymphoma cell lines, Shan *et al.*, 1998
15 *Blood* 91:1644-52, and cross-linking of CD20 on the cell surface has been reported to increase the magnitude and enhance the kinetics of signal transduction, for example, as detected by measuring tyrosine phosphorylation of cellular substrates. Deans *et al.*, 1993
J. Immunol. 146:846-53. Therefore, in addition to cellular depletion by complement and
ADCC mechanisms, Fc-receptor binding by CD20 monoclonal antibodies *in vivo* may
20 promote apoptosis of malignant B cells by CD20 cross-linking, consistent with the theory that effectiveness of CD20 therapy of human lymphoma in a SCID mouse model may be dependent upon Fc-receptor binding by the CD20 monoclonal antibody. Funakoshi *et al.*,
1996 *J. Immunotherapy* 19:93-101. The presence of multiple membrane spanning domains in the CD20 polypeptide (Einfeld *et al.*, 1988 *EMBO J.* 7:711-17; Stamenkovic *et al.*, 1988
25 *J. Exp. Med.* 167:1975-80; Tedder *et al.*, 1988 *J. Immunol.* 141:4388-4394), prevent CD20 internalization after antibody binding, and this was recognized as an important feature for therapy of B cell malignancies when a murine CD20 monoclonal antibody, 1F5, was injected into patients with B cell lymphoma, resulting in significant depletion of malignant cells and partial clinical responses. Press *et al.*, 1987 *Blood* 69:584-91.

30 Because normal mature B cells also express CD20, normal B cells are depleted by anti-CD20 antibody therapy. Reff, M.E. *et al.*, 1994 *Blood* 83:435-445. After treatment is completed, however, normal B cells can be regenerated from CD20 negative B

cell precursors; therefore, patients treated with anti-CD20 therapy do not experience significant immunosuppression. Depletion of normal B cells may also be beneficial in diseases that involve inappropriate production of autoantibodies or other diseases where B cells may play a role. A chimeric monoclonal antibody specific for CD20, consisting of heavy and light chain variable regions of mouse origin fused to human IgG1 heavy chain and human kappa light chain constant regions, reportedly retained binding to CD20 and the ability to mediate ADCC and to fix complement. Liu *et al.*, 1987 *J. Immunol.* 139:3521-26. The mechanism of anti-tumor activity of rituximab, discussed above, is thought to be a combination of several activities, including ADCC, complement fixation, and triggering of signals that promote apoptosis in malignant B cells, although the large size of rituximab prevents optimal diffusion of the molecule into lymphoid tissues that contain malignant B cells, thereby limiting these anti-tumor activities. Autoimmune diseases include autoimmune thyroid diseases, which include Graves' disease and Hashimoto's thyroiditis. In the United States alone, there are about 20 million people who have some form of autoimmune thyroid disease. Autoimmune thyroid disease results from the production of autoantibodies that either stimulate the thyroid to cause hyperthyroidism (Graves' disease) or destroy the thyroid to cause hypothyroidism (Hashimoto's thyroiditis). Stimulation of the thyroid is caused by autoantibodies that bind and activate the thyroid stimulating hormone (TSH) receptor. Destruction of the thyroid is caused by autoantibodies that react with other thyroid antigens. Current therapy for Graves' disease includes surgery, radioactive iodine, or antithyroid drug therapy. Radioactive iodine is widely used, since antithyroid medications have significant side effects and disease recurrence is high. Surgery is reserved for patients with large goiters or where there is a need for very rapid normalization of thyroid function. There are no therapies that target the production of autoantibodies responsible for stimulating the TSH receptor. Current therapy for Hashimoto's thyroiditis is levothyroxine sodium, and therapy is usually lifelong because of the low likelihood of remission. Suppressing therapy has been shown to shrink goiters in Hashimoto's thyroiditis, but no therapies that reduce autoantibody production to target the disease mechanism are known.

Rheumatoid arthritis (RA) is a chronic disease characterized by inflammation of the joints, leading to swelling, pain, and loss of function. RA affects an estimated 2.5 million people in the United States. RA is caused by a combination of events including an

initial infection or injury, an abnormal immune response, and genetic factors. While autoreactive T cells and B cells are present in RA, the detection of high levels of antibodies that collect in the joints, called rheumatoid factor, is used in the diagnosis of RA. Current therapy for RA includes many medications for managing pain and slowing the progression of the disease. No therapy has been found that can cure the disease. Medications include nonsteroidal antiinflammatory drugs (NSAIDS), and disease modifying antirheumatic drugs (DMARDS). NSAIDS are useful in benign disease, but fail to prevent the progression to joint destruction and debility in severe RA. Both NSAIDS and DMARDS are associated with significant side effects. Only one new DMARD, Leflunomide, has been approved in over 10 years. Leflunomide blocks production of autoantibodies, reduces inflammation, and slows progression of RA. However, this drug also causes severe side effects including nausea, diarrhea, hair loss, rash, and liver injury.

Systemic Lupus Erythematosus (SLE) is an autoimmune disease caused by recurrent injuries to blood vessels in multiple organs, including the kidney, skin, and joints. SLE is estimated to affect over 500,000 people in the United States. In patients with SLE, a faulty interaction between T cells and B cells results in the production of autoantibodies that attack the cell nucleus. These include anti-double stranded DNA and anti-Sm antibodies. Autoantibodies that bind phospholipids are also found in about half of SLE patients, and are responsible for blood vessel damage and low blood counts. Immune complexes accumulate the kidneys, blood vessels, and joints of SLE patients, where they cause inflammation and tissue damage. No treatment for SLE has been found to cure the disease. NSAIDS and DMARDS are used for therapy depending upon the severity of the disease. Plasmapheresis with plasma exchange to remove autoantibodies can cause temporary improvement in SLE patients. There is general agreement that autoantibodies are responsible for SLE, so new therapies that deplete the B cell lineage, allowing the immune system to reset as new B cells are generated from precursors, would offer hope for long lasting benefit in SLE patients.

Sjogren's syndrome is an autoimmune disease characterized by destruction of the body's moisture-producing glands. Sjogren's syndrome is one of the most prevalent autoimmune disorders, striking up to an estimated 4 million people in the United States. About half of people stricken with Sjogren's syndrome also have a connective tissue disease, such as RA, while the other half have primary Sjogren's syndrome with no other

concurrent autoimmune disease. Autoantibodies, including anti-nuclear antibodies, rheumatoid factor, anti-fodrin, and anti-muscarinic receptor are often present in patients with Sjogren's syndrome. Conventional therapy includes corticosteroids, and additional more effective therapies would be of benefit.

5 Immune thrombocytopenic purpura (ITP) is caused by autoantibodies that bind to blood platelets and cause their destruction. Drugs cause some cases of ITP, and others are associated with infection, pregnancy, or autoimmune disease such as SLE. About half of all cases are classified as "idiopathic", meaning the cause is unknown. The treatment of ITP is determined by the severity of the symptoms. In some cases, no therapy
10 is needed although in most cases immunosuppressive drugs, including corticosteroids or intravenous infusions of immune globulin to deplete T cells, are provided. Another treatment that usually results in an increased number of platelets is removal of the spleen, the organ that destroys antibody-coated platelets. More potent immunosuppressive drugs, including cyclosporine, cyclophosphamide, or azathioprine are used for patients with
15 severe cases. Removal of autoantibodies by passage of patients' plasma over a Protein A column is used as a second line treatment in patients with severe disease. Additional more effective therapies are desired.

 Multiple sclerosis (MS) is also an autoimmune disease. It is characterized by inflammation of the central nervous system and destruction of myelin, which insulates
20 nerve cell fibers in the brain, spinal cord, and body. Although the cause of MS is unknown, it is widely believed that autoimmune T cells are primary contributors to the pathogenesis of the disease. However, high levels of antibodies are present in the cerebral spinal fluid of patients with MS, and some theories predict that the B cell response leading to antibody production is important for mediating the disease. No B cell depletion
25 therapies have been studies in patients with MS, and there is no cure for MS. Current therapy is corticosteroids, which can reduce the duration and severity of attacks, but do not affect the course of MS over time. New biotechnology interferon (IFN) therapies for MS have recently been approved but additional more effectiver therapies are desired.

 Myasthenia Gravis (MG) is a chronic autoimmune neuromuscular disorder
30 that is characterized by weakness of the voluntary muscle groups. MG effects about 40,000 people in the united states. MG is caused by autoantibodies that bind to acetylcholine receptors expressed at neuromuscular junctions. The autoantibodies reduce

or block acetylcholine receptors, preventing the transmission of signals from nerves to muscles. There is no known cure for mg. Common treatments include immunosuppression with corticosteroids, cyclosporine, cyclophosphamide, or azathioprine. Surgical removal of the thymus is often used to blunt the autoimmune response.

- 5 Plasmapheresis, used to reduce autoantibody levels in the blood, is effective in mg, but is short-lived because the production of autoantibodies continues. Plasmapheresis is usually reserved for severe muscle weakness prior to surgery. New and effective therapies would be of benefit.

- 10 Psoriasis affects approximately five million people, and is characterized by autoimmune inflammation in the skin. Psoriasis is also associated with arthritis in 30% (psoriatic arthritis). Many treatments, including steroids, uv light retinoids, vitamin d derivatives, cyclosporine, methotrexate have been used but it is also plain that psoriasis would benefit from new and effective therapies. Scleroderma is a chronic autoimmune disease of the connective tissue that is also known as systemic sclerosis. Scleroderma is
- 15 characterized by an overproduction of collagen, resulting in a thickening of the skin, and approximately 300,000 people in the United States have scleroderma, which would also benefit from new and effective therapies.

- There is a clear need for improved compositions and methods to treat malignancies, including B cell malignancies and disorders including autoimmune diseases, disorders, and conditions, as well as other diseases, disorders, and conditions.. The compositions and methods of the present invention described and claimed herein provide such improved compositions and methods as well as other advantages.
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SUMMARY OF THE INVENTION

- It is an aspect of the present invention to provide a binding domain-immunoglobulin fusion protein, comprising a binding domain polypeptide that is fused or
- 25 otherwise connected to an immunoglobulin hinge or hinge-acting region polypeptide, which in turn is fused or otherwise connected to a region comprising one or more native or engineered constant regions from an immunoglobulin heavy chain, other than CH1, for example, the CH2 and CH3 regions of IgG and IgA, or the CH3 and CH4 regions of IgE.
- 30 The binding domain-immunoglobulin fusion protein further comprises a region that comprises, consists essentially of, or consists of, a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide (or CH3 in the case of a construct derived in

whole or in part from IgE) that is fused or otherwise connected to the hinge region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide (or CH4 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE). Such binding domain-immunoglobulin fusion proteins are capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation. Such binding domain polypeptides are also capable of binding or specifically binding to a target, for example, a target antigen.

In certain embodiments, for example, the binding domain polypeptide comprises at least one immunoglobulin variable region polypeptide that is selected from a native or engineered immunoglobulin light chain variable region polypeptide and/or a native or engineered immunoglobulin heavy chain variable region polypeptide. In certain further embodiments the binding domain-immunoglobulin fusion protein comprises a native or engineered immunoglobulin heavy chain variable region polypeptide, wherein the heavy chain variable region polypeptide is an engineered human immunoglobulin heavy chain variable region polypeptide (or an engineered immunoglobulin heavy chain variable region polypeptide from a non-human species) comprising a mutation, substitution, or deletion of an amino acid(s) at a location corresponding to any one or more of amino acid positions 9, 10, 11, 12, 108, 110, and/or 112. Mutations, substitutions, or deletions of an amino acid(s) at a location corresponding to any one or more of amino acid positions 9, 10, 11, 12, 108, 110, and/or 112 in a heavy chain variable region may be included within a construct such as the construct corresponding to, for example, SEQ ID NO: ___. In certain other further embodiments the fusion protein comprises a polypeptide having a sequence selected from SEQ ID NOS: __ or SEQ ID NO: ___. In certain embodiments the immunoglobulin variable region polypeptide is derived from, for example, a human immunoglobulin, and in certain other embodiments the immunoglobulin variable region polypeptide comprises a humanized immunoglobulin polypeptide sequence. In certain embodiments the immunoglobulin variable region polypeptide, whether or not humanized, is derived from a murine immunoglobulin, or is derived from an immunoglobulin from another species, including, for example a rat, a pig, a monkey, or a camelid.

According to certain embodiments of the present invention, the binding domain polypeptide comprises, consists essentially of, or consists of, (a) at least one native or engineered immunoglobulin light chain variable region polypeptide; (b) at least one native or engineered immunoglobulin heavy chain variable region polypeptide; and (c) at least one linker polypeptide that is fused or otherwise connected to the polypeptide of (a) and to the polypeptide of (b). In certain further embodiments the native or engineered immunoglobulin light chain variable region and heavy chain variable region polypeptides are derived or constructed from human immunoglobulins, and in certain other further embodiments the linker polypeptide comprises at least one polypeptide including or having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO: ____]. In other embodiments the linker polypeptide comprises at least two or three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO: ____]. In other embodiments the linker comprises a glycosylation site, which in certain further embodiments is an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site. In another embodiment at least one of a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide is derived from an IgG or IgA human immunoglobulin heavy chain. In another embodiment at least one of a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide and a native or engineered immunoglobulin heavy chain CH4 constant region polypeptide is derived from an IgE human immunoglobulin heavy chain.

An immunoglobulin hinge region polypeptide may comprise, consist essentially or, or consist of, for example, any of (1) any hinge or hinge-acting peptide or polypeptide that occurs naturally for example, a human immunoglobulin hinge region polypeptide including, for example, a wild-type human IgG hinge or a portion thereof, a wild-type human IgA hinge or a portion thereof, a wild-type human IgD hinge or a portion thereof, or a wild-type human IgE hinge-acting region, *i.e.*, IgE CH2, or a portion thereof, a wild-type camelid hinge region or a portion thereof (including a IgG1 llama hinge region or portion thereof, a IgG2 llama hinge region or portion thereof, and a IgG3 llama hinge region or portion thereof), a nurse shark hinge region or portion thereof, and/or a spotted ratfish hinge region or a portion thereof; (2) a mutated or otherwise altered or engineered hinge region polypeptide that contains no cysteine residues and that is derived or

constructed from a wild-type immunoglobulin hinge region polypeptide having one or more cysteine residues; (3) a mutated or otherwise altered or engineered hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type immunoglobulin hinge region polypeptide having one or more cysteine residues; (4) a
5 hinge region polypeptide that has been mutated or otherwise altered or engineered to contain or add one or more glycosylation sites, for example, an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site; (5) a mutated or otherwise altered or engineered hinge region polypeptide in which the number of cysteine residues is reduced by amino acid substitution
10 or deletion, for example, a mutated or otherwise altered or engineered IgG1 or IgG4 hinge region containing for example zero, one, or two cysteine residues, a mutated or otherwise altered or engineered IgG2 hinge region containing for example zero, one, two or three cysteine residues, a mutated or otherwise altered or engineered IgG3 hinge region containing for example zero, one, two, three, or from four to ten cysteine residues, or a
15 mutated or otherwise altered or engineered human IgA1 or IgA2 hinge region polypeptide that contains zero or only one or two cysteine residues (*e.g.*, an "SCC" hinge), a mutated or otherwise altered or engineered IgD hinge region containing no cysteine residues, or a mutated or otherwise altered or engineered human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide that contains zero or only one, two, three or four cysteine residues; or
20 (6) any other connecting region molecule described or referenced herein or otherwise known or later discovered as useful for connecting adjoining immunoglobulin domains such as, for example, a CH1 domain and a CH2 domain. For example, a hinge region polypeptide may be selected from the group consisting of (i) a wild-type human IgG1 immunoglobulin hinge region polypeptide, for example, (ii) a mutated or otherwise altered
25 or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived or constructed from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 or other immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated, (iii) a mutated or otherwise
30 altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 or other immunoglobulin hinge

region polypeptide contains no more than one cysteine residue, and (iv) a mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide contains no cysteine residues. In certain embodiments, for example, the immunoglobulin hinge region polypeptide is a mutated or otherwise altered or engineered hinge region polypeptide and exhibits a reduced ability to dimerize, relative to a wild-type human immunoglobulin G or other wild type hinge region or hinge-acting polypeptide.

The immunoglobulin heavy chain constant region polypeptides may be, for example, native or engineered CH2 and CH3 domains of an isotype that is human IgG or human IgA. The immunoglobulin heavy chain constant region polypeptides may also be, for example, native or engineered immunoglobulin heavy chain constant region CH3 and CH4 polypeptides of an isotype that is human IgE.

In certain other embodiments the target or target antigen may be, for example, CD19 (B-lymphocyte antigen CD19, also referred to as B-lymphocyte surface antigen B4, or Leu-12), CD20 (B-lymphocyte antigen 20, also referred to as B-lymphocyte surface antigen B1, Leu-16, or Bp35), CD22 (B-cell receptor CD22, also referred to as Leu-14, B-lymphocyte cell adhesion molecule, or BL-CAM), CD37 (leukocyte antigen CD37), CD40 (B-cell surface antigen CD40, also referred to as Tumor Necrosis Factor receptor superfamily member 5, CD40L receptor, or Bp50), CD80 (T lymphocyte activation antigen CD80, also referred to as Activation B7-1 antigen, B7, B7-1, or BB1), CD86 (T lymphocyte activation antigen CD86, also referred to as Activation B7-2 antigen, B70, FUN-1, or BU63), CD137 (also referred to as Tumor Necrosis Factor receptor superfamily member 9), CD152 (also referred to as cytotoxic T-lymphocyte protein 4 or CTLA-4), CD45 (Leukocyte common antigen, also referred to as L-CA, T200, and EC 3.1.3.48), CD45RA (an isoform of CD45, and an antigen expressed on naïve or immature lymphocytes), CD45RB (an isoform of CD45), CD45RO (an isoform of CD45, and a common leukocyte antigen expressed on memory B and T cells), L6 (Tumor-associated antigen L6, also referred to as Transmembrane 4 superfamily member 1, Membrane component surface marker 1, or M3S1), CD2 (T-cell surface antigen CD2, also referred to as T-cell surface antigen T11/Leu-5, LFA-2, LFA-3 receptor, Erythrocyte receptor, or

Rosette receptor), CD28 (T-cell-specific homodimer surface protein CD28, also referred to as Tp44), CD30 (lymphocyte activation antigen CD30, also referred to as Tumor Necrosis Factor receptor superfamily member 8, CD30L receptor, or Ki-1), CD50 (also referred to as Intercellular adhesion molecule-3 (ICAM3), or ICAM-R), CD54 (also referred to as Intercellular adhesion molecule-1 (ICAM1), or Major group rhinovirus receptor), B7-H1 (ligand for an immunoinhibitory receptor expressed by activated T cells, B cells, and myeloid cells, also referred to as PD-L1; see Dong, *et al.*, "B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion," 1999 *Nat. Med.* 5:1365-1369), CD134 (also referred to as Tumor Necrosis Factor receptor superfamily member 4, OX40, OX40L receptor, ACT35 antigen, or TAX-transcriptionally activated glycoprotein 1 receptor), 41BB (4-1BB ligand receptor, T-cell antigen 4-1BB, or T-cell antigen ILA), CD153 (also referred to as Tumor Necrosis Factor ligand superfamily member 8, CD30 ligand, or CD30-L), CD154 (also referred to as Tumor Necrosis Factor ligand superfamily member 5, CD40 ligand, CD40-L, TNF-related activation protein, TRAP, or T cell antigen Gp39), ICOS (Inducible Costimulator), CD3 (one or more of the delta, epsilon, gamma, eta and/or zeta chains, alone or in combination), CD4 (T-cell surface glycoprotein CD4, also referred to as T-cell surface antigen T4/Leu-3), CD25 (also referred to as Interleukin-2 receptor alpha chain, IL-2 receptor alpha subunit, p55, or Tac antigen), CD8 α (T-cell surface glycoprotein CD8 alpha chain, also referred to as T-lymphocyte differentiation antigen, T8/Leu-2, and Lyt-2), CD11b (also referred to as Integrin alpha-M, Cell surface glycoprotein MAC-1 alpha subunit, CR-3 alpha chain, Leukocyte adhesion receptor Mo1, or Neutrophil adherence receptor), CD14 (Monocyte differentiation antigen CD14, also referred to as Myeloid cell-specific leucine-rich glycoprotein or LPS receptor), CD56 (also referred to as Neural cell adhesion molecule 1), or CD69 (also referred to as Early T-cell activation antigen p60, Gp32/28, Leu-23, MLR-3, Activation inducer molecule, or AIM), and TNF factors (for example TNF- α). The above list of construct targets and/or target antigens is exemplary only and is not exhaustive.

In another aspect, the invention includes a binding construct (or a polynucleotide encoding such a construct) that comprises a CD154 extracellular domain, or desired functional portion thereof. In one embodiment of this aspect of the invention, for example, the binding construct comprises a CD154 extracellular domain fused or otherwise connected to a second binding domain. The second binding domain, for example, may

comprise, consist essentially of, or consist of at least one immunoglobulin variable region polypeptide. The at least one immunoglobulin variable region polypeptide may be a native or engineered scFv. The native or engineered scFv may be a native or engineered scFv disclosed or described herein. The second binding domain, including a native or engineered scFv, may be one that binds, for example, to any of the targets, including target antigens, disclosed or described herein, including but not limited to, for example, any of B7-H1, ICOS, L6, CD2, CD3, CD8, CD4, CD11b, CD14, CD19, CD20, CD22, CD25, CD28, CD30, CD37, CD40, CD45, CD50, CD54, CD56, CD69, CD80, CD86, CD134, CD137, CD152, CD153, or CD154.

10 In another embodiment the binding domain polypeptide comprises a CTLA-4 extracellular domain, or desired functional portion thereof, and in further embodiments at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgG1 constant region polypeptide, either native or engineered.

15 In another further embodiment at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgA constant region polypeptide, either native or engineered.

20 In another further embodiment at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH3 constant region polypeptide and a CH4 constant region polypeptide is a human IgE constant region polypeptide, either native or engineered.

Turning to another embodiment, the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide; (b) a native or engineered immunoglobulin heavy chain CH2 (or IgE Ch3) constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 (or IgE CH4) constant region polypeptide that is fused or otherwise connected to the CH2 (or IgE CH3) constant region polypeptide, wherein (1) the binding domain polypeptide comprises a CTLA-4 extracellular domain, or a portion thereof, that is capable of binding or specifically binding to at least one CTLA-4 ligand selected from the

group consisting of CD80 and CD86, (2) the immunoglobulin hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide that is selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a native or engineered human IgG1 hinge region polypeptide, and a native or engineered human IgE CH2 region polypeptide (3) a immunoglobulin heavy chain constant region polypeptide that comprises, consists essentially of, or consists of, a polypeptide that is selected from the group consisting of a native or engineered human IgA heavy chain CH2 constant region polypeptide, a native or engineered human IgG1 heavy chain CH2 constant region polypeptide, and a native or engineered human IgE heavy chain CH3 constant region polypeptide (4) a immunoglobulin heavy chain constant region polypeptide that comprises, consists essentially of, or consists of, a polypeptide that is selected from the group consisting of a native or engineered human IgA heavy chain CH3 constant region polypeptide, a native or engineered human IgG1 heavy chain CH3 constant region polypeptide, and a native or engineered human IgE heavy chain CH4 constant region polypeptide, and (5) the binding domain-immunoglobulin fusion protein is capable of inducing at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation. In a further embodiment, the binding domain-immunoglobulin fusion protein is capable of inducing two immunological activities selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation.

In another embodiment the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a native or engineered human IgE hinge-acting region, *i.e.*, a IgE CH2 region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgE CH3 constant region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or

otherwise connected to the first native or engineered constant region polypeptide, wherein said native or engineered second constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgE CH4 constant region polypeptide and wherein (1) the binding domain-immunoglobulin fusion protein is capable of inducing at least one immunological activity selected from antibody dependent cell-mediated cytotoxicity and induction of an allergic response mechanism, and (2) the binding domain polypeptide is capable of binding or specifically binding to an antigen. In a further embodiment the antigen is a tumor antigen.

In certain other embodiments the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of binding or specifically binding to at least one antigen that is present on an immune effector cell and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a native or engineered human IgG hinge region polypeptide, and a native or engineered human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said first native or engineered constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, and a native or engineered human IgE CH3 constant region polypeptide; (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first constant region polypeptide, wherein said second constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, and a native or engineered human IgE CH4 constant region polypeptide; and (d) a native or engineered plasma membrane anchor domain polypeptide. In one example of this embodiment, the plasma membrane anchor domain polypeptide links to a membrane

via a native or engineered glycosyl-phosphatidylinositol-linkage. In a further embodiment the plasma membrane anchor domain polypeptide comprises, consists essentially of, or consists of, a native or engineered transmembrane domain polypeptide. In another further embodiment the membrane anchor domain polypeptide comprises, consists essentially of, or consists of, a native or engineered transmembrane domain polypeptide and a native or engineered cytoplasmic tail polypeptide. In a still further embodiment the cytoplasmic tail polypeptide comprises, consists essentially of, or consists of, a native or engineered apoptosis signaling polypeptide sequence, which in a still further embodiment is derived or constructed from a native or engineered receptor death domain polypeptide, a death domain, or a functional portion of either. In a further embodiment the native or engineered death domain polypeptide comprises, consists essentially of, or consists of, for example, a native or engineered polypeptide selected from an ITIM domain (immunoreceptor Tyr-based inhibition motif), an ITAM domain (immunoreceptor Tyr-based activation motif), TRAF, RIP, CRADD, FADD (Fas-associated death domain), TRADD (Tumor Necrosis Factor receptor type 1 associated DEATH domain protein), RAIDD (also referred to as RAID), CD95 (Tumor Necrosis Factor receptor superfamily member 6, also referred to as FASL receptor, Apoptosis-mediating surface antigen FAS, FAS and Apo-1 antigen), TNFR1, and/or DR5 (death receptor-5). In another embodiment the native or engineered apoptosis signaling polypeptide sequence comprises, consists essentially of, or consists of, for example, a polypeptide sequence derived from a native or engineered caspase polypeptide that is caspase-3 or caspase-8 or caspase-10, including caspase 8/FLICE/MACH/Mch5 and caspase 10/Flice2/Mch4. In another embodiment the plasma membrane anchor domain polypeptide comprises, consists essentially of, or consists of, for example, a native or engineered glycosyl-phosphatidylinositol-linkage polypeptide sequence. In another embodiment the antigen that is present on an immune effector cell is, for example, CD2, CD16, CD28, CD30, CD32, CD40, CD50, CD54, CD64, CD80, CD86, B7-H1, CD134, CD137, CD152, CD153, CD154, ICOS, CD19, CD20, CD22, CD37, L6, CD3, CD4, CD25, CD8, CD11b, CD14, CD56, or CD69. In another embodiment the human IgG is a native or engineered human IgG1. These binding domain-immunoglobulin fusion proteins may be capable of inducing, for example, at least one immunological activity selected from antibody dependent cell-mediated cytotoxicity and/or complement fixation and/or CDC, and are capable of binding or specifically binding to a target,

including, for example, a target antigen. In other embodiments, the binding domain-immunoglobulin fusion proteins may be capable of inducing, for example, two immunological activities selected from antibody dependent cell-mediated cytotoxicity and/or complement fixation and/or CDC, and are capable of binding or specifically binding to a target. Immune effector cells include, for example, granulocytes, mast cells, monocytes, macrophages, dendritic cells, neutrophils, eosinophils, basophils, NK cells, T cells (including Th1 cells, Th2 cells, Tc cells, memory T cells, null cells, and large granular lymphocytes, *etc.*), and B cells. This embodiment of the invention further includes the use of such proteins for therapy, and, for example, the use of such vectors for *in vivo* and *ex vivo* gene therapy. The above lists of construct components and targets are not exhaustive, and may include any desired target or component that may function as, or be useful for the purposes, described herein.

In another embodiment, the invention provides a protein having a first protein motif that comprises, consists essentially of, or consists of, (1) a native or engineered immunoglobulin hinge region or hinge-acting region (*e.g.*, IgE CH2) polypeptide that is fused or otherwise connected to (2) a native or engineered CH2 constant region polypeptide (or native or engineered IgE CH3 constant region polypeptide). Said first protein motif may be fused or otherwise connected to one or more other such first protein motifs to form a second protein motif, the second protein motif being fused or otherwise connected to (3) a native or engineered CH3 constant region (or a native or engineered IgE CH4 constant region) to form a third protein motif. Said first, second or third protein motifs may be fused or otherwise connected to one or more of the herein-described native or engineered plasma membrane anchor domain polypeptides, including, for example, a native or engineered transmembrane domain polypeptide, and a native or engineered transmembrane domain polypeptide and a native or engineered cytoplasmic tail polypeptide, such as for example, a native or engineered apoptosis signaling polypeptide sequence, which may be derived or constructed from a native or engineered receptor death domain polypeptide, a death domain, or a functional portion of either. Thus, a protein or polynucleotide within this aspect of the invention may be, for example, a Hinge-CH2-CH3-TransmembraneDomain-DeathDomain construct. It may also be, for example, a (Hinge-CH2)_x-CH3-TransmembraneDomain-DeathDomain construct, where X is from 2 to about 5, or such other number as may be needed to achieve a desired length or Fc

receptor binding and/or complement fixation function(s). This embodiment of the invention also includes polynucleotides encoding such proteins, vectors including such polynucleotides, and host cells containing such polynucleotides and vectors. This embodiment of the invention further includes the use of such proteins for therapy, and, for example, the use of such polynucleotides and/or vectors for *in vivo* and *ex vivo* gene therapy. The invention provides, in another embodiment, a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of binding or specifically binding to at least one antigen that is present on a cancer cell surface and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a native or engineered human IgG hinge region polypeptide, and a native or engineered human IgE hinge-acting region, *i.e.*, IgE CH2, region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein the first constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is a native or engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, or a native or engineered human IgE CH3 constant region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first constant region polypeptide, wherein the second constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, or a native or engineered human IgE CH4 constant region polypeptide. In a further embodiment the human IgG polypeptides are native or engineered human IgG1 polypeptides.

In another embodiment the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide may be as described

above or herein, and may comprises, consist essentially of, or consist of, for example, a wild-type or engineered human IgA hinge region polypeptide; (b) a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered CH2 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgA CH2 constant region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise connected to the native or engineered CH2 constant region polypeptide, wherein the native or engineered CH3 constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is (i) a wild-type human IgA CH3 constant region polypeptide or other IgA region, preferably human or humanized, that is capable of associating with J Chain, (ii) a mutated, altered or otherwise engineered human IgA CH3 constant region polypeptide that is, for example, incapable of associating with a J chain, wherein (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target such as, for example, an antigen. In certain further embodiments the mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain is (i) a polypeptide comprising, consisting essentially of, or consisting of, an amino acid sequence as set forth in SEQ ID NO:___ or (ii) a polypeptide comprising, consisting essentially of, or consisting of, an amino acid sequence as set forth in SEQ ID NO:___ . In other embodiments, the IgA hinge region polypeptide is a native or engineered IgA1 hinge region polypeptide or a native or engineered IgA2 hinge region polypeptide. In still other embodiments, the IgA hinge region polypeptide is different from a wild-type IgA1 or IgA2 hinge region polypeptide by, for example, the alteration, substitution, or deletion of one or more of the cysteine residues within said wild-type hinge region.

In certain other embodiments the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide; (b) a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the

hinge region polypeptide, wherein the native or engineered CH2 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered llama CH2 constant region polypeptide that is a native or engineered llama IgG1 CH2 constant region polypeptide, a native or engineered llama IgG2 CH2 constant region polypeptide, or
5 a native or engineered llama IgG3 CH2 constant region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise connected to the native or engineered CH2 constant region polypeptide, wherein said native or engineered CH3 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered llama CH3 constant region polypeptide that is
10 selected from the group consisting of a native or engineered llama IgG1 CH3 constant region polypeptide, a native or engineered llama IgG2 CH3 constant region polypeptide and a native or engineered llama IgG3 CH3 constant region polypeptide wherein (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated
15 cytotoxicity, fixation of complement and CDC, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target, for example a target antigen. In a further embodiment the immunoglobulin hinge region polypeptide, the native or engineered llama CH2 constant region polypeptide and the native or engineered llama CH3 constant region polypeptide comprise sequences derived from a native or engineered llama
20 IgG1 polypeptide and the fusion protein does not include a native or engineered llama IgG1 CH1 domain. In certain embodiments the invention provides any of the above described binding domain-immunoglobulin fusion proteins wherein the hinge region polypeptide is mutated, engineered, or otherwise altered to contain a glycosylation site, which in certain further embodiments is an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation
25 site.

In certain embodiments the invention, there are provided any of the above or herein described binding constructs, including binding domain-immunoglobulin fusion proteins, wherein a binding region or binding domain polypeptide comprises two or more
30 binding domain polypeptide sequences wherein each of the binding domain polypeptide sequences is capable of binding or specifically binding to a target(s) such as an antigen(s), which target(s) or antigen(s) may be the same or may be different. A native, for more

preferably an engineered, IgD hinge is a desired connecting region between binding domains of a bispecific molecule of the invention, *i.e.*, one with two or more binding domains, preferably two. The wild type human IgD hinge has one cysteine that forms a disulfide bond with the light chain in the native IgD structure. It is desirable to mutate or delete this cysteine in the human IgD hinge for use as a connecting region between binding domains of, for example, a bispecific molecule. Other amino acid changes or deletions or alterations in an IgD hinge that do not result in undesired hinge inflexibility are within the scope of the invention. Native or engineered IgD hinge regions from other species are also within the scope of the invention, as are humanized native or engineered IgD hinges from non-human species. The present invention also provides, in certain embodiments, a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, an alternative hinge region polypeptide sequence; (b) a first native or engineered immunoglobulin heavy chain constant region, such as an IgG or IgA CH2 constant region polypeptide (or an IgE CH3 constant region polypeptide) that is fused or otherwise connected to the hinge region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region, such as an IgG or IgA CH3 constant region polypeptide (or an IgE CH4 constant region polypeptide) that is fused or otherwise connected to the first constant region polypeptide, wherein: (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target, such as an antigen.

Turning to another embodiment there is provided a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of binding or specifically binding to at least one target, such as an antigen, that is present on a cancer cell surface and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, an alternative hinge region

polypeptide sequence; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, and a native or engineered human IgE CH3 constant region polypeptide; and (c) a second immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first constant region polypeptide, wherein the second constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, or a native or engineered human IgE CH4 constant region polypeptide. In certain further embodiments the alternative hinge region polypeptide sequence comprises, consists essentially of, or consists of, a polypeptide sequence of at least ten continuous amino acids that are present in a sequence selected from SEQ ID NOS: __-__.

In certain embodiments the present invention provides polynucleotides or vectors (including cloning vectors and expression vectors) or transformed or transfected cells, including isolated or purified or pure polynucleotides, vectors, and isolated transformed or transfected cells, encoding or containing any one of the above or herein described polypeptide or protein constructs of the invention, for example, including binding domain-immunoglobulin fusion proteins. Thus, in various embodiments the invention provides a recombinant cloning or expression construct comprising any such polynucleotide that is operably linked to a promoter.

In other embodiments there is provided a host cell transformed or transfected with, or otherwise containing, any such recombinant cloning or expression construct. Host cells include the cells of a subject undergoing *ex vivo* cell therapy including, for example, *ex vivo* gene therapy.

In a related embodiment there is provided a method of producing a polypeptide or protein or other construct of the invention, for example, including a binding domain-immunoglobulin fusion protein, comprising the steps of (a) culturing a host cell as described or provided for herein under conditions that permit expression of the construct, for example, a binding domain-immunoglobulin fusion protein; and (b) isolating the

construct, for example, the binding domain-immunoglobulin fusion protein from the host cell or host cell culture.

In another embodiment there is provided a pharmaceutical composition comprising any one of the above or herein described polypeptide or protein or other
5 constructs of the invention, for example (including, for example, binding domain-immunoglobulin fusion proteins), in combination with a physiologically acceptable carrier.

In another embodiment the invention provides a pharmaceutical composition comprising, for example, an isolated, purified, or pure polynucleotide encoding any one of the polypeptide or protein constructs of the invention, for example
10 (including, for example, binding domain-immunoglobulin fusion proteins), in combination with a physiologically acceptable carrier, or for example, in combination with, or in, a gene therapy delivery vehicle or vector.

In another embodiment the invention provides a method of treating a subject having or suspected of having a malignant condition or a B cell disorder, comprising
15 administering to a patient a therapeutically effective amount of any of the pharmaceutical compositions described or claimed herein.

In certain further embodiments the malignant condition or B cell disorder is a B cell lymphoma or B cell leukemia, or a disease characterized by autoantibody production, and in certain other further embodiments the B cell disorder is, for example,
20 rheumatoid arthritis, myasthenia gravis, Grave's disease, type I diabetes mellitus, multiple sclerosis or an autoimmune disease. In certain other embodiments the malignant condition is, for example, melanoma, myeloma, glioma, astrocytoma, lymphoma, leukemia, carcinoma, or sarcoma, and so on.

It is another aspect of the present invention to provide a binding domain-immunoglobulin fusion protein, comprising, consisting essentially or, or consisting of, (a) a
25 binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide is as described herein, and may be selected from the group consisting of (i) a mutated, engineered or otherwise altered hinge region polypeptide that contains no cysteine residues and that is derived from a wild-type immunoglobulin hinge region polypeptide having one or more cysteine residues, (ii) a
30 mutated, engineered or otherwise altered hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type immunoglobulin hinge region

polypeptide having two or more cysteine residues, (iii) a wild-type human IgA hinge region polypeptide, (iv) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains no cysteine residues, (v) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains one cysteine residue and (vi) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains two cysteine residues; (b) a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise connected to the CH2 constant region polypeptide, wherein: (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to an antigen. In one embodiment the immunoglobulin hinge region polypeptide is a mutated hinge region polypeptide, for example, and the resulting construct exhibits a reduced ability to dimerize, relative to a construct containing a wild-type human immunoglobulin G hinge region polypeptide. In another embodiment the binding domain polypeptide comprises, consists essentially of, or consists of, at least one native or engineered immunoglobulin variable region polypeptide that is a native or engineered immunoglobulin light chain variable region polypeptide and/or a native or engineered immunoglobulin heavy chain variable region polypeptide. In a further embodiment the native or engineered immunoglobulin variable region polypeptide is derived from a human immunoglobulin and, for example, may be humanized.

In another embodiment, the invention provides a binding domain-immunoglobulin fusion protein includes a binding domain polypeptide that comprises, consists essentially of, or consists of, (a) at least one native or engineered immunoglobulin light chain variable region polypeptide; (b) at least one native or engineered immunoglobulin heavy chain variable region polypeptide; and (c) at least one linker peptide that is fused or otherwise connected to the polypeptide of (a) and to the polypeptide of (b). In a further embodiment the native or engineered immunoglobulin light chain variable region and the native or engineered heavy chain variable region polypeptides are derived from human immunoglobulins and may, for example, be humanized.

In another embodiment at least one of the native or engineered immunoglobulin heavy chain CH2 (or IgE CH3) constant region polypeptide and the native or engineered immunoglobulin heavy chain CH3 (or IgE CH4) constant region polypeptide is derived or constructed from a human immunoglobulin heavy chain. In another embodiment the native or engineered immunoglobulin heavy chain constant region CH2 and CH3 polypeptides are of, or are derived or otherwise prepared or constructed from, an isotype selected from human IgG and human IgA. In another embodiment the target, for example, the target antigen is selected from the group consisting of CD16, CD19, CD20, CD37, CD40, CD45RO, CD80, CD86, CD137, CD152, and L6. In certain further embodiments of the above described fusion protein construct, the binding domain comprises, consists essentially of, or consists of, an scFv and the scFv contains a linker polypeptide that comprises, consists essentially of, or consists of, at least one polypeptide comprising or having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:___], and in certain other embodiments the linker polypeptide comprises, consists essentially of, or consists of, at least three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:___]. In certain embodiments the immunoglobulin hinge region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgG, IgA, IgD hinge region polypeptide, or a native or engineered IgE CH2 region polypeptide. In certain embodiments the binding domain polypeptide comprises, consists essentially of, or consists of, a native or engineered CD154 extracellular domain. In certain embodiments the binding domain polypeptide comprises, consists essentially of, or consists of, a native or engineered CD154 extracellular domain and at least one a native or engineered immunoglobulin variable region polypeptide.

In other embodiments the invention provides an isolated polynucleotide encoding any of the constructs of the invention, for example, protein or polypeptide constructs of the invention including binding domain-immunoglobulin fusion proteins, and in related embodiments the invention provides a recombinant expression construct comprising such a polynucleotide, and in certain further embodiments the invention provides a host cell transformed or transfected with, or otherwise containing, such a recombinant expression construct. In another embodiment the invention provides a method of producing a construct of the invention, for example, a protein or polypeptide construct of the invention such as a binding domain-immunoglobulin fusion protein, comprising the

steps of (a) culturing a host cell that has been transformed or transfected with, or otherwise made to contain, a polynucleotide construct of the invention under conditions that permit expression of the construct, for example, a construct encoding a binding domain-immunoglobulin fusion protein; and (b) isolating the construct, for example, the binding domain-immunoglobulin fusion protein, from the host cell culture.

The inventions described and claimed herein include novel molecules useful, for example, as therapeutics and other purposes including diagnostic and research purposes. Such molecules have, for example, antigen binding or other binding function(s) and one or more effector functions. DNA constructs of the invention are useful in, for example, gene therapies, including *in vivo* and *ex vivo* gene therapies.

In one aspect, various constructs of the molecules of the invention include molecules comprising a "binding region", a "tail" region, and a "connecting" region that joins a binding region and a tail region.

Binding regions within the molecules of the invention may comprise, for example, binding domains for desired targets, including antigen-binding targets. Binding domains for antigen-binding targets may comprise, for example, single chain Fvs and scFv domains. In certain embodiments, molecules of the invention may comprise a binding region having at least one immunoglobulin variable region polypeptide, which may be a light chain or a heavy chain variable region polypeptide. In certain embodiments, molecules of the invention may comprise at least one such light chain V-region and one such heavy chain V-region and at least one linker peptide that connects the V-regions. ScFvs useful in the invention also include those with chimeric binding or other domains or sequences. Other ScFvs useful in the invention also include those with humanized binding or other domains or sequences. In such embodiments, all or a portion of an immunoglobulin binding or other sequence that is derived from a non-human source may be "humanized" according to recognized procedures for generating humanized antibodies, *i.e.*, immunoglobulin sequences into which human Ig sequences are introduced to reduce the degree to which a human immune system would perceive such proteins as foreign.

Example of scFvs useful in the invention, whether included as murine or other scFvs (including human scFvs), chimeric scFvs, or humanized scFvs, in whole or in part, include anti-human CD20 scFvs (for example, "2H7" scFvs), anti-human CD37 scFvs (for example, "G28-1" scFvs), anti-human CD40 scFvs (for example, "G28-5" scFvs and

“40.2.220” scFvs), anti-carcinoma-associated antigen scFvs (for example, “L6” scFvs), anti-CTLA-4 (CD152) scFvs (for example, “10A8” scFvs), anti-human CD28 scFvs (for example, “2E12” scFvs), anti-murine CD3 scFvs (for example, “500A2” scFvs), anti-human CD3 scFvs (for example, G19-4 scFvs), anti-murine 4-1BB scFvs (for example, “1D8” scFvs), anti-human 4-1BB scFvs (for example, “5B9” scFvs), anti-human CD45RO (for example, “UCHL-1” scFvs), and anti-human CD16 (for example, “Fc2” scFvs).

scFvs useful in the invention also include scFvs, including chimeric and humanized scFvs, having one or more amino acid substitutions. A preferred amino acid substitution is at amino acid position 11 in the variable heavy chain (the V_H). Such a substitution may be referred to herein as “Xxx V_H 11Zxx”. Thus, for example, where the normally occurring amino acid at position V_H 11 is a Leucine, and a Serine amino acid residue is substituted therefore, the substitution is identified as “L V_H 11S” or “Leu V_H 11Ser.” Other preferred embodiments of the invention include molecules containing scFvs wherein the amino acid residue normally found at position V_H 11 is deleted. Still other preferred embodiments of the invention include molecules containing scFvs wherein the amino acid residues normally found at positions V_H 10 and/or V_H 11 and/or V_H 12 are substituted or deleted.

Other binding regions within the molecules of the invention may include domains that comprise sites for glycosylation, for example, covalent attachment of carbohydrate moieties such as monosaccharides or oligosaccharides.

Still other binding regions within molecules of the invention include polypeptides that may comprise proteins or portions thereof that retain the ability to specifically bind another molecule, including an antigen. Thus, binding regions may comprise or be derived from hormones, cytokines, chemokines, and the like; cell surface or soluble receptors for such polypeptide ligands; lectins; intercellular adhesion receptors such as specific leukocyte integrins, selectins, immunoglobulin gene superfamily members, intercellular adhesion molecules (ICAM-1, -2, -3) and the like; histocompatibility antigens; and so on. Binding regions derived from such molecules generally will include those portions of the molecules necessary or desired for binding to a target.

Certain constructs include binding regions that comprise receptor or receptor-binding domains. Receptor domains useful for binding to a target include, for example, a CD154 extracellular domain, or a CTLA-4 extracellular domain. In another

example, the binding domain may include a first portion comprising, consisting essentially or, or consisting of, a CD154 extracellular domain and a second portion comprising, consisting essentially or, or consisting of, at least one immunoglobulin variable region polypeptide, said second portion including, for example, an scFv or a V_H. Examples of other cell surface receptors that may comprise, consist essentially or, or consist of, or a portion of which may provide, a binding region or binding domain polypeptide, include, for example, HER1, HER2, HER3, HER4, epidermal growth factor receptor (EGFR), vascular endothelial cell growth factor, vascular endothelial cell growth factor receptor, insulin-like growth factor-I, insulin-like growth factor-II, transferrin receptor, estrogen receptor, progesterone receptor, follicle stimulating hormone receptor (FSH-R), retinoic acid receptor, MUC-1, NY-ESO-1, Melan-A/MART-1, tyrosinase, Gp-100, MAGE, BAGE, GAGE, any of the CTA class of receptors including in particular HOM-MEL-40 antigen encoded by the SSX2 gene, carcinoembryonic antigen (CEA), and PyLT. Additional cell surface receptors that may be sources of binding regions or binding domain polypeptides include, for example, CD2, 4-1BB, 4-1BB ligand, CD5, CD10, CD27, CD28, CD152/CTLA-4, CD40, interferon- γ (IFN- γ), interleukin-4 (IL-4), interleukin-17 (IL-17) and interleukin-17 receptor (IL-17R). Still other cell surface receptors that may be sources of binding regions and/or binding domain polypeptides include, for example, CD59, CD48, CD58/LFA-3, CD72, CD70, CD80/B7.1, CD86/B7.2, B7-H1/B7-DC, IL-17, CD43, ICOS, CD3 (*e.g.*, gamma subunit, epsilon subunit, delta subunit), CD4, CD25, CD8, CD11b, CD14, CD56, CD69 and VLA-4 ($\alpha_4\beta_7$). The following cell surface receptors are typically associated with B cells: CD19, CD20, CD22, CD30, CD153 (CD30 ligand), CD37, CD50 (ICAM-3), CD106 (VCAM-1), CD54 (ICAM-1), interleukin-12, CD134 (OX40), CD137 (41BB), CD83, and DEC-205. These lists are not exhaustive. Binding regions such as those set forth above may be connected, for example, by a native or engineered IgD hinge region polypeptide, preferably a human or humanized native or engineered IgD hinge region polypeptide. The invention thus further provides constructs that comprise, consist essentially of, or consist of, two binding regions, for example, an scFv and a cell surface receptor (or portion thereof), connected by a third molecule, for example, an IgD hinge region polypeptide as described herein.

Various molecules of the invention described and claimed herein include a connecting region joining one end of the molecule to another end. Such connecting

regions may comprise, for example, immunoglobulin hinge region polypeptides, including any hinge peptide or polypeptide that occurs naturally. A connecting region may also include, for example, any artificial peptide or other molecule (including, for example, non-peptide molecules, partial peptide molecules, and peptidomimetics, *etc.*) useful for joining

5 the tail region and the binding region. These may include, for example, alterations of molecules situated in an immunoglobulin heavy chain polypeptide between the amino acid residues responsible for forming intrachain immunoglobulin-domain disulfide bonds in CH1 and CH2 regions. Naturally occurring hinge regions include those located between the constant region domains, CH1 and CH2, of an immunoglobulin. Useful

10 immunoglobulin hinge region polypeptides include, for example, human immunoglobulin hinge region polypeptides and llama or other camelid immunoglobulin hinge region polypeptides. Other useful immunoglobulin hinge region polypeptides include, for example, nurse shark and spotted ratfish immunoglobulin hinge region polypeptides. Human immunoglobulin hinge region polypeptides include, for example, wild type IgG

15 hinges including wild-type human IgG1 hinges, human IgG-derived immunoglobulin hinge region polypeptides, a portion of a human IgG hinge or IgG-derived immunoglobulin hinge region, wild-type human IgA hinge region polypeptides, human IgA-derived immunoglobulin hinge region polypeptides, a portion of a human IgA hinge region polypeptide or IgA-derived immunoglobulin hinge region polypeptide, wild-type human

20 IgD hinge region polypeptides, human Ig-D derived immunoglobulin hinge region polypeptides, a portion of a human IgD hinge region polypeptide or IgD-derived immunoglobulin hinge region polypeptide, wild-type human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptides (which generally have 5 cysteine residues), human IgE-derived immunoglobulin hinge region polypeptides, a portion of a human IgE hinge-acting

25 region, *i.e.*, IgE CH2 region polypeptide or IgE-derived immunoglobulin hinge region polypeptide, and so on. A polypeptide "derived from" or that is "a portion or fragment of" an immunoglobulin polypeptide chain region regarded as having hinge function has one or more amino acids in peptide linkage, for example 15-115 amino acids, preferably 95-110, 80-94, 60-80, or 5-65 amino acids, preferably 10-50, more preferably 15-35, still more

30 preferably 18-32, still more preferably 20-30, still more preferably 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acids. Llama immunoglobulin hinge region polypeptides include, for example, an IgG1 llama hinge. The connecting region may comprise a stretch of

consecutive amino acids from an immunoglobulin hinge region. For example, the connecting region can comprise at least five consecutive hinge region amino acids, at least ten consecutive hinge region amino acids, at least fifteen consecutive hinge region amino acids, at least 20 consecutive hinge region amino acids, and at least twenty five or more consecutive hinge region amino acids from human IgG hinge, human IgA hinge, human IgE hinge, camelid hinge region, IgG1 llama hinge region, nurse shark hinge region, and spotted ratfish hinge region, including for example an IgG₁ hinge region, a IgG₂ hinge region, a IgG₃ hinge region, an IgG₃ hinge region, and an IgG₄ hinge region.

Such connecting regions also include, for example, mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides. A mutated or otherwise altered or engineered immunoglobulin hinge region polypeptide may comprise, consist essentially of, or consist of, a hinge region that has its origin in an immunoglobulin of a species, of an immunoglobulin isotype or class, or of an immunoglobulin subclass that is the same or different from that of any included native or engineered CH2 and CH3 domains. Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, a wild-type immunoglobulin hinge region that contains one or more cysteine residues, for example, a wild-type human IgG or IgA hinge region that naturally comprises three cysteines. In such polypeptides the number of cysteine residues may be reduced by amino acid substitution or deletion or truncation, for example. These polypeptides include, for example, mutated human or other IgG1 or IgG4 hinge region polypeptides containing zero, one, or two cysteine residues, and mutated human or other IgA1 or IgA2 hinge region polypeptides that contain zero, one, or two cysteine residues. Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, a wild-type immunoglobulin hinge region that contains three or more cysteine residues, for example, a wild-type human IgG2 hinge region (which has 4 cysteines) or IgG4 hinge region (which has 11 cysteines). Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, an IgE CH2 wild-type immunoglobulin region that generally contains five cysteine residues. In such polypeptides the number of cysteine residues may be reduced by one or more cysteine residues by amino acid substitution or deletion or truncation, for example. Also included are an altered hinge region polypeptides in which cysteine

residues in the hinge region are substituted with serine or one or more other amino acids that are less polar, less hydrophobic, more hydrophilic, and/or neutral. Such mutated immunoglobulin hinge region polypeptides include, for example, mutated hinge region polypeptides that contain one cysteine residue and that are derived from a wild-type immunoglobulin hinge region polypeptide having two or more cysteine residues, such as a mutated human IgG or IgA hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type human IgG or IgA region polypeptide. Connecting region polypeptides include immunoglobulin hinge region polypeptides that are compromised in their ability to form interchain, homodimeric disulfide bonds.

Mutated immunoglobulin hinge region polypeptides also include mutated hinge region polypeptides that exhibit a reduced ability to dimerize, relative to a wild-type human immunoglobulin G hinge region polypeptide, and mutated hinge region polypeptides that allow expression of a mixture of monomeric and dimeric molecules. Mutated immunoglobulin hinge region polypeptides also include hinge region polypeptides engineered to contain a glycosylation site. Glycosylation sites include, for example, an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site, and a phosphoglycation site.

Specific connecting regions useful in molecules of the invention described and claimed herein include, for example, the following 18 amino acid sequences, DQEPKSCDKTHTCPPCPA, DQEPKSSDKTHTSPPPSPA, and DLEPKSCDKTHTCPPCPA. Other specific connecting regions include, for example, the mutant hinges within the sequences referred to herein as "2H7 scFv (SSS-S)H WCH2 WCH3" and "2H7 scFv (CSS)H WCH2 WCH3", and the human IgA-derived hinge referred to herein as "2H7 scFv IgAH WCH2 WCH3".

Tail regions within the molecules of the invention may include heavy chain constant region immunoglobulin sequences. Tail regions may thus include, for example, a polypeptide having at least one of an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide. At least one of the immunoglobulin heavy chain CH2 constant region polypeptide and the immunoglobulin heavy chain CH3 constant region polypeptide may be derived from a human immunoglobulin heavy chain. Thus, for example, CH2 and/or CH3 polypeptides may be derived from human IgG, human IgA, or human IgD molecules. Tail regions may

also include, for example, a polypeptide having at least one of an immunoglobulin heavy chain CH3 constant region polypeptide and an immunoglobulin heavy chain CH4 constant region polypeptide. At least one of the immunoglobulin heavy chain CH3 constant region polypeptide and the immunoglobulin heavy chain CH4 constant region polypeptide may be derived from a human immunoglobulin heavy chain. Thus, for example, CH3 and/or CH4 polypeptides may be derived from human IgE. An immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. An immunoglobulin heavy chain CH3 region polypeptide included within a molecule of the invention may also, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. Additionally, both the immunoglobulin heavy chain CH2 region polypeptide and the immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. In other molecules of the invention at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgA constant region polypeptide. An immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgA1 and/or IgA2 subclasses. An immunoglobulin heavy chain CH3 region polypeptide included within a molecule of the invention may also, for example, be from the IgA1 and/or IgA2 subclasses. Additionally, both the immunoglobulin heavy chain CH2 region polypeptide and the immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgA1 and/or IgA2 subclasses. In still other molecules of the invention, the tail region may comprise or consist essentially of a CH2 and/or CH3 constant region polypeptide comprising a polypeptide from human IgA and/or human IgE. In other embodiments, for example, the tail region within a molecule of the invention may include an immunoglobulin heavy chain CH2 and/or CH3 constant region polypeptide that is a mutated (for example, a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain in which, for example, the IgA CH3 constant region polypeptide is of human origin). The tail region may also comprise, consist essentially of, or consist of an extracellular portion of a protein from the TNF superfamily, for example, CD154.

For molecules of the invention intended for use in humans, these regions will typically be substantially or completely human to minimize potential human immune responses against the molecules and to provide appropriate effector functions. In certain embodiments of the invention, for example, the tail region includes a human IgG1 CH3
5 region sequence, a wild-type IgA heavy chain constant region polypeptide sequence that is capable or incapable of associating with J chain.

In preferred embodiments of the invention, a CH1 domain is not included in the tail region of the molecule, and the carboxyl end of the binding region is joined to the amino terminus of a CH2 portion of a tail region either directly or indirectly. A binding
10 region may be indirectly joined to a tail region, for example via a connecting region polypeptide or other connecting molecule.

The invention also includes molecules that have mutated CH2 and/or CH3 sequences within a tail region. For example, a molecule of the invention may include a mutated Fc domain that has one or more mutations introduced into the CH2, CH3 and/or
15 CH4 domains. In certain embodiments of the invention, molecules may include an IgA CH3 constant region polypeptide such as a human IgA CH3 constant region polypeptide in which two or more residues from the C-terminus have been deleted to yield a truncated CH3 constant region polypeptide. In other embodiments of the invention, molecules include a mutated human IgA CH3 constant region polypeptide that is incapable of
20 associating with a J chain that comprises a C-terminal deletion of either four or 18 amino acids. However, the invention need not be so limited, such that molecules containing the mutated IgA CH3 constant region polypeptide may comprise a deletion of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30 or more amino acids, so long as the fusion protein is capable of specifically binding an antigen and capable of at least one
25 immunological activity such as ADCC, CDC or complement fixation. The invention also includes molecules containing a tail region that comprises a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain by virtue of replacement of the penultimate cysteine, or by chemical modification of that amino acid residue, in a manner that prevents interchain disulfide bond formation.

30 Various molecules of the invention include, for example, a binding domain scFv- fusion protein having a binding domain polypeptide comprising, consisting essentially of, or consisting of, (a) at least one immunoglobulin light chain variable region

polypeptide, (b) at least one immunoglobulin heavy chain variable region polypeptide, and at least one linker peptide that joins the polypeptide of (a) and the polypeptide of (b). Such polypeptides may, for example, be derived from human immunoglobulins or non-human immunoglobulins.

5 Thus, in one aspect, the invention includes a non-naturally occurring single chain protein and/or V_H protein and/or V_L protein, or a desired portion of any of the above, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a flexible or other desired linker attached to said first polypeptide, a third polypeptide comprising a tail region, for example,
10 an N-terminally truncated immunoglobulin heavy chain constant region polypeptide (or desired portion thereof) attached to the second polypeptide. The flexible linker may comprise, consist essentially of, or consist of, an immunoglobulin hinge region or portion thereof that has been mutated or otherwise altered or engineered, for example, one that contains a number of cysteine residues that is less than the number of cysteine residues
15 present in the wild type immunoglobulin hinge region or portion (for example, zero, one, or two cysteines in the case of IgG1 or IgG4), and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity, for example, ADCC, CDC, and/or complement fixation. The single chain protein may be capable of two immunological activities including, for example, ADCC, CDC, and/or complement
20 fixation. This protein may include a binding domain polypeptide that is a single chain Fv. Additionally, this protein may include a binding domain polypeptide that is a single chain Fv wherein the heavy chain variable region of the single chain Fv has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112. The protein may also include a binding domain polypeptide that is a single chain Fv
25 wherein the light chain variable region of the single chain Fv has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107.

 In another aspect, the invention includes a non-naturally occurring V_H protein, or a desired portion thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule or construct, a V_H region or portion
30 thereof that has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112 of said V_H region. Amino acids may be

substituted with either naturally occurring or non-naturally occurring amino acids, or any other desired useful molecule.

Also described and claimed are uses of V_H proteins, or desired portions thereof, that comprise, consist essentially of, or consist of, alone or in combination with
5 any other molecule or construct, a V_H region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112 of said V_H region. Such uses include uses in phage display, yeast display, and ribosome display systems and methods.

In yet another aspect, the invention includes a non-naturally occurring V_L
10 protein, or a desired portion thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule, a V_L region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 of said V_L region. Amino acids may be substituted with either naturally occurring or non-naturally occurring amino acids, or any other desired useful molecule.

Also described and claimed are uses of V_L proteins, or desired portions
15 thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule, a V_L region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 of said V_L region. Such uses include uses in phage display, yeast display, and ribosome
20 display systems and methods.

In yet another aspect, the invention includes a molecule comprising, consisting essentially of, or consisting of, (1) a V_H protein, or a desired portion thereof, wherein the V_H protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112, and (2) a non-naturally
25 occurring V_L protein, or a desired portion thereof, alone or in combination with any other molecule, wherein the V_L protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107. Amino acids may be substituted with either naturally occurring or non-naturally occurring amino acids, or any other desired useful molecule.

Also described and claimed are uses of a molecule comprising, consisting
30 essentially of, or consisting of, (1) a V_H protein, or a desired portion thereof, wherein the V_H protein or portion thereof has an amino acid deletion or substitution at one or more of

amino acid positions 9, 10, 11, 12, 108, 110, and 112, and (2) a non-naturally occurring V_L protein, or a desired portion thereof, alone or in combination with any other molecule, wherein the V_L protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107. Such uses include uses
5 in phage display, yeast display, and ribosome display systems and methods.

The invention also includes molecular constructs wherein the binding domain is a single chain Fv and the heavy chain variable region of said single chain Fv has an amino acid substitution at amino acid position 11. The amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region may be
10 selected from the group consisting of serine, threonine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine. The invention thus includes, for example, a construct wherein the binding domain is a single chain Fv and the heavy chain variable region of said single chain Fv has a serine amino acid substitution at amino acid position 11. Other amino acid position changes, substitutions, and deletions, are noted
15 herein.

The invention also includes, for example, a construct wherein the binding domain is a single chain Fv and the amino acid at position 10 and/or 11 of the heavy chain variable region of said single chain Fv has been deleted.

In another aspect, the invention includes constructs wherein the binding
20 region binds to a tumor or tumor-associated antigen. The binding region of a construct of the invention may bind, for example, to a cancer cell antigen. Cancer cell antigens to which constructs of the invention bind include cancer cell surface antigens and intracellular cancer cell antigens.

In yet another aspect, the invention includes a construct wherein the binding
25 region binds to an antigen on an immune effector cell.

In another aspect, the invention includes a construct wherein the binding region binds to a B cell antigen including, for example, a B cell antigen selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86. Constructs of the invention that bind to such B cell antigens include, for example, binding regions
30 comprising an single chain Fv. Examples of such single chain Fv binding regions include molecules comprising or consisting essentially of single chain Fvs selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and

4.4.220 single chain Fv. Other examples include a binding region comprising, consisting essentially of, or consisting of, an extracellular domain of CTLA-4.

In another aspect, the invention includes a construct wherein the binding region binds to a B cell differentiation antigen. B cell differentiation antigens include, for example, CD19, CD20, CD21, CD22, CD23, CD37, CD40, CD45RO, CD80, CD86, and HLA class II.

In another aspect, the invention includes a construct wherein the binding region binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

The invention also includes protein constructs having a binding region, a tail region, and a connecting region, wherein the protein construct is capable of existing in solution as a monomer or in substantially monomeric form.

The invention also includes protein constructs having a binding region, a tail region, and a connecting region, wherein the protein construct is capable of forming a complex comprising two or more of said protein constructs including, for example, wherein said complex is a dimer.

In another aspect, constructs of the invention are capable of participating in or inducing or eliciting or helping to induce or elicit, directly or indirectly, at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity (or complement-mediated lysis), complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

In another aspect, binding constructs of the invention are capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components. Such biologically active molecules are, for example, proteases.

Other biologically active molecules are, for example, cytokines, including by way of example monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.

5 In another aspect, constructs of the invention are capable of induction, or participation in the induction, of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

10 In another aspect, constructs of the invention are capable of induction, or participation in the induction, of one or more immune effector cells that results in antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

In another aspect, constructs of the invention are capable of participating in and/or initiating apoptosis within target cells, for example, by activating one or more signalling mechanisms or molecules.

15 In another aspect, constructs of the invention are capable of induction, or participation in the induction, of cellular activation, wherein said activation leads to changes in cellular transcriptional activity. In one embodiment, cellular transcriptional activity is increased. In another embodiment, cellular transcriptional activity is decreased.

20 In another aspect, constructs of the invention having tail regions comprising, consisting essentially of, or consisting of, constant regions from IgA or IgE molecules, are capable of induction, or participation in the induction, of degranulation of neutrophils and/or mast cells.

25 In another aspect, constructs of the invention are capable of promotion, or participation in the promotion, of neutralization of an infectious agent, wherein said infectious agent is, for example, a bacterium, a virus, a parasite, or a fungus.

30 In another aspect, constructs of the invention are capable of promoting, or participating in the promotion of, neutralization of a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins. Such toxins include, for example, exotoxins selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF toxin. Other toxins include, for example, saxitoxins,

tetrodotoxin, mushroom toxins (amatoxins, gyromitrin, orellanine, *etc.*), aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.

In another aspect, constructs of the invention are capable of binding to an intracellular target to, for example, effect (or participate in effecting) a cellular function.

5 Such constructs include, for example, constructs that include a tail region comprising, consisting essentially of, or consisting of, a native or engineered IgA CH2 domain region and a native or engineered IgA CH3 domain region, said tail region being capable of binding J chain. Such a tail region is found, for example, in the 2H7 scFv IgAH WlgACH2 WCH3 + JChain construct. Thus, the invention includes constructs having, for example,
10 an "Anti-Intracellular Target" binding domain (for example, and "Anti-Intracellular Target" scFv), a connecting region, and a native or engineered IgA constant region capable of binding J chain (for example, WlgACH2 WCH3).

In still another aspect, constructs of the invention include a molecule wherein an N-terminally immunoglobulin heavy chain constant region polypeptide
15 comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

In yet another aspect, the invention includes a method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein molecule that is less than about 120kK, or less than about
20 150kD, as measured, for example, by HPLC and non-reducing gels and consists essentially of (a) a first protein or peptide molecule that is capable of binding to cells within said target cell population, and (b) a second protein or peptide molecule that is capable of (i) binding to an Fc receptor and/or (ii) inducing target cell apoptosis, and/or (iii) fixing complement, wherein said first protein or peptide molecule is directly connected to said
25 second protein or peptide molecule, or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope.

30 In another aspect, the invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second

polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of at least one immunological activity, and provided that (a) when the connecting region polypeptide comprises an IgG hinge region polypeptide having no cysteine residues, the binding domain polypeptide target is not CD20 or L6, or (b) when the connecting region polypeptide comprises an IgG hinge region polypeptide having no cysteine residues, the single chain protein is not a 1F5 scFv capable of binding to CD20. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of binding to a target molecule on or in a target cell and decreasing the number of target cells *in vivo* and/or depleting a population of target cells *in vivo*. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of inducing antibody-dependent cell-mediated cytotoxicity and complement fixation. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of (1) inducing antibody-dependent cell-mediated cytotoxicity and complement fixation, and (2) binding to a target molecule on or in a target cell and decreasing the number of target cells

in vivo and/or depleting a population of target cells *in vivo*. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is substituted or deleted, and wherein said single-chain protein is capable of at least one immunological activity. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is substituted or deleted, and wherein said single-chain protein is capable of inducing at least one immunological activity selected from (a) antibody-dependent cell-mediated cytotoxicity and (b) complement fixation. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is

substituted or deleted, and wherein said single-chain protein is capable of antibody-dependent cell-mediated cytotoxicity and complement fixation. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is substituted or deleted, and wherein said single-chain protein is capable of binding to a target molecule on or in a target cell and decreasing the number of target cells *in vivo* and/or depleting a population of target cells *in vivo*. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is substituted or deleted, and wherein said single-chain protein is capable of inducing at least one immunological activity selected from antibody-dependent cell-mediated cytotoxicity and complement fixation, and wherein said single-chain protein is capable of binding to a target molecule on or in a target cell and decreasing the number of target cells *in vivo* and/or depleting a population of target cells *in vivo*. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated

immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is substituted or deleted, and wherein said single-chain protein is capable of inducing antibody-dependent cell-mediated cytotoxicity and complement fixation, and wherein said single-chain protein is capable of binding to a target molecule on or in a target cell and decreasing the number of target cells *in vivo* and/or depleting a population of target cells *in vivo*. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine at position 11 in the first framework region of the heavy chain variable region is deleted or substituted with another amino acid; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of at least one immunological activity, and provided that when the binding domain polypeptide is capable of binding to CD20 said connecting region comprises three cysteine residues wherein one or two of said three cysteine residues is substituted or replaced with another amino acid. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine at position 11 in the first framework region of the heavy chain variable region is deleted or substituted with another amino acid, (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of inducing at least one immunological activity, provided that when binding domain polypeptide is a 2H7 scFv capable of binding to CD20 and said connecting region comprises an IgG hinge region

polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or two of said cysteine residues is substituted or deleted. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of,

5 (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule on or in a target cell, said binding domain polypeptide comprising a heavy chain variable region wherein leucine at position 11 in the first framework region of the heavy chain variable region is deleted or substituted with another amino acid; (ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and (iii) a

10 third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said single-chain protein is capable of at least one immunological activity, provided that said single chain protein does not comprise, or consist essentially of, a binding domain polypeptide capable of binding to CD20 and a connecting region that comprises (a) an IgG hinge having three

15 cysteine residues or (b) an IgG hinge comprising three serine (or like amino acid) residues that have been substituted for cysteine residues. There are many possible variations and variants of these single chain proteins. For example, the binding domain polypeptide may be a single chain antibody or scFv including naturally occurring and/or non-naturally occurring V_H and V_L polypeptides, and the binding domain polypeptide may bind any of a

20 number of targets. Non-naturally occurring V_H polypeptides include, by way of example and not limitation, human heavy chain variable region polypeptide comprising a mutation, substitution, or deletion of an amino acid(s) at a location corresponding to any one or more of amino acid positions 9, 10, 11, 12, 108, 110, and/or 112. Non-naturally occurring V_L polypeptides include, by way of example and not limitation, human light chain variable

25 region polypeptides comprising a mutation, substitution, or deletion of an amino acid(s) at a location corresponding to any one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107. Targets include, by way of example and not limitation, CD19, CD20, CD28, CD30, CD37, CD40, L6, HER2, epidermal growth factor receptors (EGFRs), vascular endothelial cell growth factors (VEGFs), tumor necrosis factors (*e.g.*, TNF-alpha), as well

30 as other targets described or referred to herein or otherwise useful, whether now known or later discovered. Additionally, the connecting region polypeptide may be any of a number of molecules, both naturally occurring and non-naturally occurring. Connecting region

polypeptides include, by way of example and not limitation, naturally occurring and non-naturally occurring immunoglobulin hinge region polypeptides. Naturally occurring immunoglobulin hinge region polypeptides include wild-type immunoglobulin hinge region polypeptides such as, by way of example and not limitation, human IgG1 hinge
5 region polypeptides, human IgA hinge region polypeptides, human IgD hinge region polypeptides, human IgE hinge-acting regions (*e.g.*, IgE CH2), camelid immunoglobulin hinge regions, or any other naturally occurring hinge region peptides described or referred to herein or otherwise useful, whether now known or later discovered. Non-naturally occurring immunoglobulin hinge region polypeptides include, by way of example
10 and not limitation, mutated naturally occurring immunoglobulin hinges, including immunoglobulin hinge region polypeptides that contain less than the wild-type number of cysteines, for example, mutated naturally occurring immunoglobulin hinge region polypeptides that contain zero, one, or two cysteines, and any other connecting region molecule described or referenced herein or otherwise useful or now known or later
15 discovered as useful for connecting joining, for example, immunoglobulin domains such as a CH1 domain and a CH2 domain. N-terminally truncated immunoglobulin heavy chain constant region polypeptides include naturally occurring and non-naturally occurring N-terminally truncated immunoglobulin heavy chain constant region polypeptides that, with or without other portions of the single chain protein, provide one or more effector functions
20 such as those described herein. Naturally occurring N-terminally truncated immunoglobulin heavy chain constant region polypeptides include, by way of example and not limitation, CH2CH3 constant region polypeptides, including CH2CH3 constant region polypeptides taken, separately or together, from human IgGs, human IgAs, and human IgE, and any other immunoglobulin heavy chain constant region polypeptide described or
25 referenced herein or otherwise now known or later discovered to be useful. Non-naturally occurring N-terminally truncated immunoglobulin heavy chain constant region polypeptides include, by way of example and not limitation, any mutated naturally occurring heavy chain constant region polypeptide described or referred to herein or otherwise now known or later discovered to be useful.

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Various specific constructs of the invention include, by way of example only, the following:

1. 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3
2. 2H7 scFv VH L11S IgE CH2 CH3 CH4
3. 2H7 scFv VH L11S mIgE CH2 CH3 CH4
4. 2H7 scFv VH L11S mIgAH WlgACH2 T4CH3
5. 2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3
6. 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3
7. 2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3
8. 2HU scFv VH L11S (CSS-S) H P331S CH2 WCH3
9. 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3
10. 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3
11. 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3
12. 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3
13. G28-1 scFv (SSS-S) H WCH2 WCH3
14. G28-1 scFv IgAH WCH2 WCH3
15. G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3
16. G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3
17. G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3
18. G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3
19. CTLA4 (SSS-S) H P238SCH2 WCH32
20. CTLA4 (CCC-P) WH WCH2 WCH3
21. FC2-2 scFv (SSS-S) H WCH2 WCH3
22. FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3
23. UCHL-1 scFv (SSS-S) H WCH2 WCH3
24. UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3
25. 5B9 scFv (SSS-S) H WCH2 WCH3
26. 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3
27. 2H7 scFv (SSS-S) H WCH2 WCH3
28. 2H7 scFv (SSS-S) H P238SCH2 WCH3
29. 2H7 scFv IgAH WCH2 WCH3
30. 2H7 scFv IgAH WlgACH2 T4CH3
31. 2H7 scFv IgAH WlgACH2 WCH3 + JChain
32. 2H7 scFv (CCC-P) WH WCH2 WCH3

33. 2H7 scFv (SSS-S) H WCH2 F405YCH3
34. 2H7 scFv (SSS-S) H WCH2 F405ACH3
35. 2H7 scFv (SSS-S) H WCH2 Y407ACH3
36. 2H4 scFv (SSS-S) HWCH2 F405A, Y407ACH3
- 5 37. 2H7 scFv (CSS-S) H WCH2 WCH3
38. 2H7 scFv (SCS-S) H WCH2 WCH3
39. 2H7 scFv (SSC-P) H WCH2 WCH3
40. 2H7 scFv (CSC-S) H WCH2 WCH3
41. 2H7 scFv (CCS-P) H WCH2 WCH3
- 10 42. 2H7 scFv (SCC-P) H WCH2 WCH3
43. 2H7 scFv VH L11S (SSS-S) H WCH2 WCH3
44. 2H7 scFv VH L11S (CSS-S) H WCH2 WCH3
45. G28-1 scFv VH L11S (SCS-S) H WCH2 WCH3
46. G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3
- 15 47. G28-1 scFv VH L11S (SCC-P) H WCH2 WCH3
48. G28-1 scFv VH L11S mlgE CH2 CH3 CH4
49. G28-1 scFv VH L11S mIgAH WlgACH2 T4CH3
50. G28-1 scFv VH L11S hIgE CH2 CH3 CH4
51. G28-1 scFv VH L11S hIgAH WlgACH2 T4CH3
- 20 52. HD37 scFv IgAH WCH2 WCH3
53. HD37 scFv (SSS-S) H WCH2 WCH3
54. HD37 scFv VH L11S (SSS-S) H WCH2 WCH3
55. L6 scFv IgAH WCH2 WCH3
56. L6 scFv VHL11S (SSS-S) H WCH2 WCH3
- 25 57. 2H7 scFv-llama IgG1
58. 2H7 scFv-llama IgG2
59. 2H7 scFv-llama IgG3
60. CD16-6 low (ED)(SSS-S) H P238SCH2 WCH3
61. CD16-9 high (ED)(SSS-S) H P238SCH2 WCH3
- 30 62. 2e12 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
63. 10A8 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
64. 40.2.36 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT

65. 2H7 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
66. G19-4 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
67. 2e12 scFv (SSS-s)H WCH2 WCH3—hCD80TM/CT
68. 2e12 scFv IgAH IgACH2 T4CH3—hCD80TM/CT
- 5 69. 2e12 scFv IgE CH2CH3CH4—hCD80TM/CT
70. 2e12 scFv (SSS-s)H P238SCH2 WCH3—mFADD-TM/CT
71. 2e12 scFv (SSS-s)H WCH2 WCH3—mFADD-TM/CT
72. 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp3-TM/CT
73. 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp3-TM/CT
- 10 74. 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp8--TM/CT
75. 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT
76. 2e12 scFv (SSS-s)H WCH2 WCH3—hcas3--TM/CT
77. 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcas3-TM/CT
78. 2e12 scFv (SSS-s)H WCH2 WCH3—hcas8--TM/CT
- 15 79. 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcas8-TM/CT
80. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
81. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hCD80TM/CT
82. 1D8 scFv—mIgAT4—hCD80TM/CT
83. 1D8 scFv--hIgE—hCD80TM/CT
- 20 84. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mFADD-TM/CT
85. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mFADD-TM/CT
86. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcasp3-TM/CT
87. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp3-TM/CT
88. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcasp8--TM/CT
- 25 89. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT
90. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcas3--TM/CT
91. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hcas3-TM/CT
92. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcas8--TM/CT
93. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hcas8-TM/CT L6
- 30 scFv (SSS-S) H WCH2 WCH3
94. 2H7 scFv CD154 (L2)
95. 2H7 scFv CD154 (S4)
96. CTLA4 IgAH IGACH2CH3

97. CTLA4 IgAH IgACH2 T4CH3

98. 2H7 scFv IgAH IgACH2CH3

99. 2H7 scFv IgAH IgAHCH2 T18CH3

5 100. 2H&-40.2.220 scFv (SSS-S) H WCH2 WCH3 (bispecific anti-ccd20-anti-cd40)

101. 2H7 scFv IgAH IgACH2 T4CH3-hCD89 TM/CT

102. G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT

103. 2e12 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT

10 These and other aspects of the present invention will become further apparent upon reference to the following detailed description and attached drawings. As noted herein, all referenced patents, articles, documents, and other materials disclosed or identified herein are hereby incorporated by reference in their entireties as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

15 **Figure 1** shows DNA and deduced amino acid sequences [SEQ ID NOS:___] of 2H7scFv-Ig, a binding domain-immunoglobulin fusion protein capable of specifically binding CD20.

Figure 2 shows production levels of 2H7 scFv-Ig by transfected, stable CHO lines and generation of a standard curve by binding of purified 2H7 scFv-Ig to CHO
20 cells expressing CD20.

Figure 3 shows SDS-PAGE analysis of multiple preparations of isolated 2H7scFv-Ig protein.

Figure 4 shows complement fixation (Fig. 4A) and mediation of antibody-dependent cellular cytotoxicity (Fig. 4B) by 2H7scFv-Ig.

25 **Figure 5** shows the effect of simultaneous ligation of CD20 and CD40 on growth of normal B cells.

Figure 6 shows the effect of simultaneous ligation of CD20 and CD40 on CD95 expression and induction of apoptosis in a B lymphoblastoid cell line.

30 **Figure 7** shows DNA and deduced amino acid sequences [SEQ ID NOS:___] of 2H7scFv-CD154 L2 (Fig. 7A, SEQ ID NOS:___) and 2H7scFv-CD154 S4 (Fig. 7B, SEQ ID NOS:___) binding domain-immunoglobulin fusion proteins capable of specifically binding CD20 and CD40.

Figure 8 shows binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion proteins to CD20+ CHO cells by flow immunocytofluorimetry.

Figure 9 shows binding of Annexin V to B cell lines Ramos, BJAB, and T51 after binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion protein to cells.

Figure 10 shows effects on proliferation of B cell line T51 following binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion protein.

Figure 11 depicts schematic representations of the structures of 2H7ScFv-Ig fusion proteins [SEQ ID NOS: __] referred to as CytoxB or CytoxB derivatives: CytoxB-MHWTG1C (2H7 ScFv, mutant hinge, wild-type human IgG1 Fc domain), CytoxB-MHMG1C (2H7 ScFv, mutant hinge, mutated human IgG1 Fc domain) and CytoxB-IgA_HWTHG1C (2H7 ScFv, human IgA-derived hinge [SEQ ID NO: __], wild-type human IgG1 Fc domain). Arrows indicate position numbers of amino acid residues believed to contribute to FcR binding and ADCC activity (heavy arrows), and to complement fixation (light arrows). Note absence of interchain disulfide bonds.

Figure 12 shows SDS-PAGE analysis of isolated CytoxB and 2H7scFv-CD154 binding domain-immunoglobulin fusion proteins.

Figure 13 shows antibody dependent cell-mediated cytotoxicity activity of CytoxB derivatives.

Figure 14 shows complement dependent cytotoxicity of CytoxB derivatives.

Figure 15 shows serum half-life determinations of CytoxB-MHWTG1C in macaque blood samples.

Figure 16 shows effects of CytoxB-MHWTG1C on levels of circulating CD40+ B cells in macaque blood samples.

Figure 17 shows production levels of HD37 (CD19-specific) ScFv-Ig by transfected mammalian cell lines and generation of a standard curve by binding of purified HD37 ScFv-Ig to cells expressing CD19.

Figure 18 shows production levels of L6 (carcinoma antigen) ScFv-Ig by transfected, stable CHO lines and generation of a standard curve by binding of purified L6 ScFv-Ig to cells expressing L6 antigen.

Figure 19 shows antibody dependent cell-mediated cytotoxicity activity of binding domain-immunoglobulin fusion proteins 2H7 ScFv-Ig, HD37 ScFv-Ig and G28-1 (CD37-specific) ScFv-Ig.

Figure 20 shows antibody dependent cell-mediated cytotoxicity activity of L6 ScFv-Ig fusion proteins.

Figure 21 shows SDS-PAGE analysis of L6 ScFv-Ig and 2H7 ScFv-Ig fusion proteins.

Figure 22 shows SDS-PAGE analysis of G28-1 ScFv-Ig and HD37 ScFv-Ig fusion proteins.

Figure 23 presents a sequence alignment of immunoglobulin hinge and CH2 domains of human IgG1 (SEQ ID NO:__) with the hinge and CH2 domains of llama IgG1 (SEQ ID NO:__), IgG2 (SEQ ID NO:__), and IgG3 (SEQ ID NO:__).

Figure 24 illustrates migration of purified 2H7 scFv llama IgG fusion proteins in a 10% SDS polyacrylamide gel. Purified fusion proteins (5 µg per sample) were prepared in non-reducing sample buffer (lanes 2-5) and in reducing sample buffer (lanes 6-9). Lane 1: molecular weight markers (non-reduced); lanes 2 and 6: 2H7 scFv-llama IgG1 (SEQ ID NO:__); Lanes 3 and 7: 2H7 scFv-llama IgG2 (SEQ ID NO:__); lanes 4 and 8: 2H7 scFv-llama IgG3 (SEQ ID NO:__); and Lanes 5 and 9: Rituximab (chimeric anti-CD20 antibody (human IgG1 constant region)).

Figure 25 shows binding of 2H7 scFv-llama IgG1 (SEQ ID NO:__), 2H7 scFv-llama IgG2 (SEQ ID NO:__), and 2H7 scFv-llama IgG3 (SEQ ID NO:__) to CD20+ CHO cells detected by flow immunocytometry.

Figure 26 depicts CDC activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv-llama IgG1 (SEQ ID NO:__), 2H7 scFv-llama IgG2 (SEQ ID NO:__), and 2H7 scFv-llama IgG3 (SEQ ID NO:__), and 2H7 scFv human IgG1 (2H7 scFv IgG WTH WTCH2CH3) (SEQ ID NO:__) against BJAB cells in the presence of rabbit complement. Rituximab was included as a control.

Figure 27 shows antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv-llama IgG1 (SEQ ID NO:__), 2H7 scFv-llama IgG2 (SEQ ID NO:__), and 2H7 scFv-llama IgG3 (SEQ ID NO:__). Effector cells (human PBMC) were combined with target cells (BJAB cells) at three different ratios,

1:25, 1:50, and 1:100. Rituximab was included as a control. Each data point represents three separate measurements.

Figure 28 shows antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv-llama IgG1 (SEQ ID NO:__), 2H7 scFv-llama IgG2 (SEQ ID NO:__), and 2H7 scFv-llama IgG3 (SEQ ID NO:__). Effector cells (llama PBMC) were combined with target cells (BJAB cells) at three different ratios, 1:25, 1:50, and 1:100. Rituximab was included as a control. Each data point represents three separate measurements.

Figure 29 depicts complement dependent cytotoxicity activity of Reh cells (acute lymphocytic leukemia) expressing scFv-Ig fusion proteins on the cell surface. Reh cells were transfected with constructs encoding scFv antibodies specific for human costimulatory molecules, CD152, CD28, CD40, and CD20, fused to human IgG1 wild-type hinge-CH2-CH3, which was fused to human CD80 transmembrane and cytoplasmic tail domains. Complement dependent cytotoxicity activity was measured in the presence and absence of rabbit complement (plus C' and no C', respectively). The data represent the average of duplicate samples. Reh anti-hCD152 scFvIg: Reh cells transfected with polynucleotide 10A8 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:__); Reh anti-hCD28scFvIg: 2E12 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:__); Reh anti-hCD40scFvIg: 4.2.220 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:__); and Reh anti-hCD20scFvIg: 2H7 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:__).

Figure 30 presents antibody dependent cell-mediated cytotoxicity activity of Reh cells that were transfected with constructs encoding scFv antibodies specific for human costimulatory molecules, CD152, CD28, CD40, and CD20, as described for Figure 29, and for murine CD3, fused to human mutant IgG1 hinge and mutant CH2 and wild type CH3 (Reh anti-mCD3scFv designating Reh cells transfected with polynucleotide 500A2 scFv IgG MTH (SSS) MTCH2WTCH3 SEQ ID NO:__), which was fused to human CD80 transmembrane and cytoplasmic tail domains. The data represent the average of quadruplicate samples.

Figure 31 lists immunoglobulin constant region constructs that were used in experiments illustrated in subsequent figures.

Figure 32 depicts complement dependent cytotoxicity activity of CTLA-4 Ig fusion proteins, CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:__) (2 µg/ml)

and CTLA-4 IgG MTH MTCH2WTCH3 (SEQ ID NO:__) (2 µg/ml), in the presence and absence of rabbit complement (plus C' and no C', respectively). The target cells were Reh cells and Reh cells transfected with CD80 (Reh CD80.10).

Figure 33 shows antibody dependent cell-mediated cytotoxicity activity of CTLA-4 Ig fusion proteins, CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:__) (2 µg/ml) and CTLA-4 IgG MTH MTCH2WTCH3 (SEQ ID NO:__) (2 µg/ml). Effector cells, human PBMC, were added to target cells, Reh or Reh CD80.1, at the ratios indicated. Figure 33A presents the level of natural killing in Reh CD80.1 cells in the absence of any Ig fusion protein. Figure 33B presents antibody dependent cell-mediated cytotoxicity mediated by CTLA-4 IgG MTH MTCH2WTCH3, and Figure 33C presents antibody dependent cell-mediated cytotoxicity mediated by CTLA-4 IgG WTH (CCC) WTCH2CH3. Each data point represents the average percent specific killing measured in four sample wells.

Figure 34 illustrates binding of 2H7 (anti-CD20) scFv Ig fusion proteins to (CD20+) CHO cells by flow immunocytofluorimetry.

Figure 35 presents an immunoblot of 2H7 scFv IgG and IgA fusion proteins. COS cells were transiently transfected with various 2H7 scFv Ig fusion protein constructs. The expressed polypeptides were immune precipitated with protein A, separated in a non-reducing SDS polyacrylamide gel, and then transferred to a polyvinyl fluoride membrane. Proteins were detected using an anti-human IgG (Fc specific) horseradish peroxidase conjugate. Lane 1: vector only; lane 2: 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:__); lane 3: 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO:__); lane 4: 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:__); lane 5: 2H7 scFv IgAH IgG WTCH2CH3 (SEQ ID NO:__); and lane 6: 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:__).

Figure 36 illustrates binding of 2H7 scFv IgAH IgACH2CH3 polypeptide (SEQ ID NO:__) and 2H7 scFv IgAH IgAT4 (SEQ ID NO:__) to (CD20+) CHO cells by flow immunocytofluorimetry. The source of the polypeptides was culture supernatants from transiently transfected COS cells. COS cells transfected with a plasmid comprising a sequence encoding 2H7 scFv IgAH IgACH2CH3 were co-transfected with a plasmid containing nucleotide sequence encoding human J chain.

Figure 37 illustrates antibody dependent cell-mediated cytotoxicity activity of anti-CD20 (2H7) scFv Ig fusion proteins against BJAB target cells using whole blood as the source of effector cells. Purified 2H7 scFv Ig fusion proteins were titrated and combined with ^{51}Cr -labeled BJAB cells (5×10^4) and whole blood (1:4 final dilution). Each data point represents the average percent specific killing measured in four sample wells.

Figure 38 demonstrates antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv Ig fusion proteins ($5 \mu\text{g/ml}$) against ^{51}Cr -labeled BJAB cells at 0.25, 0.125, and 0.625 dilutions of whole blood. Each data point represents the average percent specific killing measured in four sample wells.

Figure 39 shows a comparison of antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv IgG MTH (SSS) WTCH2CH3 ($5 \mu\text{g/ml}$) and 2H7 scFv IgAH IgACH2CH3 ($5 \mu\text{g/ml}$) when human PBMC are the source of effector cells (Figure 39A) and when human whole blood is the source of effector cells (Figure 39B).

Figure 40 presents an immunoblot of 2H7 scFv IgG fusion proteins. COS cells were transiently transfected with various 2H7 scFv Ig fusion protein constructs. Culture supernatants containing the expressed polypeptides were separated in a non-reducing SDS polyacrylamide gel, and then were transferred to a polyvinyl fluoride membrane. Proteins were detected using an anti-human IgG (Fc specific) horseradish peroxidase conjugate. Lanes 1-5: purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 at 40 ng, 20 ng, 10 ng/ 5 ng, and 2.5 ng per lane, respectively. Culture supernatants were separated in lanes 6-9. Lane 6: 2H7 scFv IgG WTH (CCC) WTCH2CH3; lane 7: 2H7 scFv IgG MTH (CSS) WTCH2CH3; lane 8: 2H7 scFv IgG MTH (SCS) WTCH2CH3; and lane 9: 2H7 scFv VHSE11 IgG MTH (SSS) WTCH2CH3. The molecular weight (kDa) of marker proteins is indicated on the left side of the immunoblot.

Figure 41 illustrates cell surface expression of 1D8 (anti-murine 4-1BB) scFv IgG WTH WTCH2CH3-CD80 fusion protein on K1735 melanoma cells by flow immunofluorimetry (Fig. 41A). The scFv fusion protein was detected with phycoerythrin-conjugated F(ab')_2 goat anti-human IgG. Fig. 41B depicts growth of tumors in naïve C3H mice transplanted by subcutaneous injection with wild type K1735 melanoma cells (K1735-WT) or with K1735 cells transfected with 1D8 scFv IgG WTH WTCH2CH3-CD80 (K1735-1D8). Tumor growth was monitored by measuring the size of the tumor.

Fig. 41C demonstrates the kinetics of tumor growth in naïve C3H mice injected intraperitoneally with monoclonal antibodies to remove CD8⁺, CD4⁺, or both CD4⁺ and CD8⁺ T cells prior to transplantation of the animals with K1735-1D8 cells.

Figure 42 demonstrates therapy of established K1735-WT tumors using K1735-1D8 as an immunogen. Six days after mice were transplanted with K1735-WT tumors, one group (five animals) was injected subcutaneously with K1735-1D8 cells (open circles) or irradiated K1735-WT cells (solid squares) on the contralateral side. A control group of mice received PBS (open squares). Treatments were repeated on the days indicated by the arrows.

Figure 43 shows the growth of tumors in animals that were injected subcutaneously with 2×10^6 K1735-WT cells (solid squares) and the growth of tumors in animals that were injected subcutaneously with 2×10^6 K1735-WT cells plus 2×10^5 K1735-1D8 cells (open triangles).

Figure 44 presents a flow cytometry analysis of antigen104 murine sarcoma tumor cells transfected with 1D8 scFv IgG WTH WTCH2CH3-CD80 isolated after repeated rounds of panning against anti-human IgG. Transfected cells expressing 1D8 scFv IgG WTH WTCH2CH3-CD80 were detected with fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG (depicted in black). Untransfected cells are shown in gray.

Figure 45 illustrates migration of various 2H7 scFv Ig fusion proteins in a 10% SDS-PAGE gel. 2H7 was the anti-CD20 scFv and 40.2.220 was the anti-CD40 scFv. Lane 1: Bio-Rad prestained molecular weight standards; lane 2: anti-CD20 scFv IgG MTH (SSS) MTCH2WTCH3; lane 3: anti-CD20 scFv IgG MTH (SSS) WTCH2CH3; lane 4: 2H7 scFv IgAH IgG WTCH2CH3; lane 5: anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3; lane 6: Rituximab; lane 7: Novex Multimark® molecular weight standards.

Figure 46 illustrates effector function as measured in an antibody dependent cell-mediated cytotoxicity assay of 2H7 Ig fusion proteins that contain a mutant CH2 domain or wild type CH2 domain. The percent specific killing of BJAB target cells in the presence of human PBMC effector cells by 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (diamonds) was compared to 2H7 scFv IgG MTH (SSS) WTCH2CH3 (squares) and 2H7 scFv IgAH IgG WTCH2CH3 (triangles) and Rituximab (circles).

Figure 47 shows cell surface expression of an anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 (SEQ ID NO:__) fusion protein on Reh cells (Fig. 47A) and T51 lymphoblastoid cells (Fig. 47B) by measuring the linear fluorescent equivalent (LFE) using flow immunocytofluorimetry.

5 **Figure 48** presents the percent specific killing of untransfected Reh and T51 cells and the percent specific killing of Reh cells (Reh anti-hCD3) (Fig. 48A) and T51 cells (T51 anti-hCD3) (Fig. 48B) that were transfected with a construct encoding scFv antibodies specific for human CD3, fused to human IgG1 wild-type hinge-CH2-CH3, which was fused to human CD80 transmembrane and cytoplasmic tail domains (anti-
10 human CD3 scFv IgG WTH WTCH2CH3-CD80 (SEQ ID NO:__). Human PBMC (effector cells) were combined with BJAB target cells at the ratios indicated.

Figure 49 illustrates binding of 5B9, an anti-murine CD137 (4-1BB) monoclonal antibody, and a 5B9 scFv IgG fusion protein (5B9 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:__) to stimulated human PBMC. Binding of the 5B9 scFv
15 IgG fusion protein was detected by flow immunocytofluorimetry using FITC conjugated goat anti-human IgG. Binding of the 5B9 monoclonal antibody was detected with FITC conjugated goat anti-mouse IgG.

Figure 50 illustrates the effect of the LV_H11S mutation on the expression of 2H7 LV_H11S scFv WCH2 WCH3 ("CytosB scFv Ig"; SEQ ID NO:__) in CHO cell lines.

20 **Figure 51** shows a semi-quantitative SDS-PAGE analysis examining the expression of 2H7 LV_H11S scFv WCH2 WCH3 (SEQ ID NO:__) when transiently transfected in CHO cells. Lanes 2-5 are various amounts of 2H7 LV_H11S scFv WCH2 WCH3. Lanes 6-10 are 10 μ l samples from five different clones expressing 2H7 LV_H11S scFv WCH2 WCH3.

25 **Figure 52** shows differences in binding capacity between a G28-1 LV_H11S scFv Ig construct (SEQ ID NO: __) and a G28-1 wild type scFv Ig binding domain fusion protein construct (SEQ ID NO:__), both obtained from transiently transfected COS cells. Binding to Ramos cells was determined using flow cytometry. The data illustrates a significant increase in binding of the V_H11S protein to CD37+Ramos cells.

30 **Figure 53** illustrates increased levels of expression of a G28-1 LV_H11S scFv Ig construct (SEQ ID NO: __) compared to a G28-1 wild type scFv Ig construct in COS. Protein levels were compared using immunoblot analysis. Both immunoblot gels

have quantitated amounts of purified a G28-1 scFv Ig (SSS-S) H WCH2 WCH3 construct of the invention in lanes 1-4. Lanes 5-9 of the first immunoblot represent five different clones each transfected with G28-1 scFv (SSS-S) H WCH2 WCH3, while lanes 5-9 of the second immunoblot represent five different clones transfected with G28-1 LV_H11S scFv (SSS-S) H WCH2 WCH3. The immunoblots illustrate that the LV_H11S form causes the G28-1 scFv Ig construct to express at very high levels.

Figure 54 illustrates the binding of 2H7 scFv Ig derivatives with altered hinges (SEQ ID NOs: __, __, __, __, __, __) to CHO cells expressing CD20 (CD20+ CHO) by flow cytometry, and indicates that these altered connecting region hinge constructs (including (SSS-S), (CSS-S), (SCS-S) and (CSC-S) hinge regions) retain binding function to CD20.

Figure 55 shows the ability to mediate antibody dependent cell-mediated cytotoxicity of various constructs against Bjab targets: (A) 2H7 scFv Ig constructs of the invention that contain connecting regions comprising (CSS-S), (SCS-S), (CSC-S), and (SSS-S) hinges (SEQ ID NOs: __, __, __, __, __, __) and (B) 2H7 scFv constructs of the invention with various connecting regions and tail regions (SEQ ID NOs: __, __, __, __, __, __). Percent specific killing is compared to total killing induced by a detergent. The controls are natural killing in target cells with effectors added and a 2H7 construct with an IgA hinge connecting region and IgA-derived tail region that does not bind PBMC effectors.

Figure 56 illustrates the ability of various 2H7 scFv Ig constructs of the invention (SEQ ID NO: __, __, __, __, __, __) that include connecting regions having various hinge regions (*e.g.*, (CSC-S), (SSS-S), (SCS-S), and (CSS-S)) to mediate complement activity in Ramos cells. Percent specific killing is measured against the control of complement only, and 100% killing was determined by exposure of cells to detergent.

Figure 57 illustrates the shows the binding of 2H7 scFv Ig constructs of the invention containing different tail regions (SEQ ID NO: __, __, __) to CD20+ CHO using immunocytofluorimetry. The different proteins were detected using FITC conjugated to anti-IgG, anti-IgA, and anti-IgE.

Figure 58A shows the binding of 2H7 V_H L11S scFv IgECH2CH3CH4, purified using Hydrophobic charge induction chromatography (HCIC) and eluted at different pHs 4.0 and 3.5, (SEQ ID NO: __) in CD20+ CHO cells by flow cytometry,

indicating that the proteins bound CD20 whether eluted at pH 4.0 or 3.5. **Figure 58B** is a data graph indicating the ability of these 2H7 VH L11S scFv IgE constructs of the invention to mediate, for example, ADCC in Bjab target cells.

Figure 59 shows the binding capacity of G28-1 VH L11S mIgECH2CH3CH4 (SEQ ID NO:) (A) to Bjab and Ramos target cells and (B) to CD20+ CHO cells by flow cytometry.

Figure 60 shows the High Performance Liquid Chromatography (HPLC) profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H (P238S)CH2 WCH3 (SEQ ID NO:) (B) 2H7 scFv (CSS-S) H WCH2 WCH3, (SEQ ID NO:) (C) 2H7 scFv (SCS-S) H WCH2 WCH3, (SEQ ID NO:) and (D) 2H7 scFv (SSS-S) H WCH2 (Y407A)CH3 (SEQ ID NO:), indicating that construct A has apparent molecular weight forms of 100kD and 75kD and that, by introducing certain changes a predominant 75kD molecular weight form is obtained, as seen in constructs B, C, and D. See Example 40.

Figure 61 shows the HPLC profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H WCH2 WCH3, (SEQ ID NO:) (B) 2H7 scFv (CSC-S) H WCH2 WCH3, (SEQ ID NO:) (C) 2H7 scFv (CCC-P) H WCH2 WCH3, (SEQ ID NO:) and (D) 2H7 scFv IgAH WCH2 WCH3 (SEQ ID NO:), indicating that construct A has apparent molecular weight forms of 100kD and 75kD and that, by introducing certain changes a predominant 75kD molecular weight form is obtained, as seen in constructs B and C, while construct D (which has an IgA tail region) has an apparent molecular weight of 150kD. See Example 40.

Figure 62 shows the HPLC profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H WCH2 WCH3, (SEQ ID NO:) (B) 2H7 scFv (SCS-S) H WCH2 WCH3, (SEQ ID NO:) (C) 2H7 scFv IgA 3TCH2 WCH3, (SEQ ID NO:) and (D) 2H7 scFv (SSS-S) H WCH2 (F405A Y407A)CH3 (SEQ ID NO:), indicating that construct A has two forms with apparent molecular weights at 100kD and 75kD, construct B has a predominant form with an apparent molecular weight of 75kD, while construct C with a T4 mutation leads to three forms with apparent molecular weights near 600kD and construct D with a double point mutation in the CH3 region leads to a predominant form having an apparent molecular weight less than 44kD. A T4 mutation here refers to a truncation of four amino acids from a CH3 region. See Example 40.

Figure 63 compares the effect on binding CD20+CHO cells by 2H7 V_H L11S scFv Ig constructs (SEQ ID NOs: __,__), with and without F405A and Y407A alterations in the CH3 region, by flow cytometry, indicating a loss of binding capability with this double amino acid change. See Example 41.

5 **Figure 64** shows the binding capacity of FITC conjugated 2H7 V_H L11S scFv Ig derivatives (SEQ ID NOs: __,__,__,__) to CH20+ CHO cells by flow cytometry, indicating that these constructs do not lose binding capacity when conjugated to a fluorescent marker. See Example 41.

10 **Figure 65** shows a nonreducing SDS-PAGE analysis examining 10 µg (per lane) of various purified 2H7 V_H L11S scFv Ig constructs of the invention (SEQ ID NOs: __,__,__,__,__,__), indicating an apparent molecular weight for each construct in reference to a standard molecular weight marker in lane 1. See Example 41.

15 **Figure 66** compares the CH2 domain sequences of four different human IgG regions, hIgG1, hIgG2, hIgG3, hIgG4, and one rat region, rIgG2b. Point mutations affecting ADCC and CDC are labeled with arrows. See Example 52.

20 **Figure 67** demonstrates the ability of various 2H7 V_H L11S scFv Ig constructs (SEQ ID NOs: __,__) to mediate ADCC in CHO and Lec13 CHO transiently transfected cells, indicating that constructs expressed in Lec 13 CHO cells had a 20% increase in specific killing over the same construct expressed in regular CHO cells. See Example 42.

Figure 68 shows SDS-PAGE analysis, both reduced and nonreduced, of high and low affinity alleles of soluble CD 16(ED) (SSS-S) H P283S CH2 WCH3 (SEQ ID NOs: __,__). See Example 43.

25 **Figure 69** demonstrates the different binding capabilities of the high and low affinity CD16 fusion proteins (SEQ ID NOs: __,__) to 2H7 V_H L11S scFv (CSC-S) WCH2 WCH3 (SEQ ID NO: __) or 2H7 V_H L11S scFv (SSS-S) WCH2 WCH3 (SEQ ID NO: __), and indicates a loss of high and low affinity allele binding using P238S CH2 constructs. See Example 43.

30 **Figure 70** shows a diagram of (A) an assay used to detect changes in Fc receptor binding using FITC conjugated CD16 extracellular domain Ig fusion protein with a mutated tail, which eliminates self-association and B) a mammalian display system using cell surface expression of constructs. See Example 44.

Figure 71 shows the induction of apoptosis in Bjab and Ramos cells by various mAbs (SEQ ID NOs: __, __, __, __, __, __) and scFvIg constructs of the invention (SEQ ID NOs: __, __, __, __, __). See Example 45.

Figure 72 illustrates the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs to induce apoptosis in Ramos cells that is mediated by activation of caspase 3. The results indicate that under these conditions, both constructs bind CD20 and induced apoptosis.

Figure 73 illustrates the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs to mediate CDC activity in CD20 positive Bjab target cells. The results indicate both constructs had the ability to mediate CDC.

Figure 74 compares the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs to mediate ADCC in Bjab target cells. The results indicate the 2H7 scFv (SSS-S) H WCH2 WCH3 construct was very effective and induced high levels of specific killing, while the of 2H7 scFv (SSS-S) H P238CH2 WCH3 construct was not effective in mediating ADCC.

Figure 75 compares the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs to bind soluble CD16 (high affinity and low affinity forms) in CD20 positive CHO cells. Results indicate that 2H7 scFv (SSS-S) H WCH2 WCH3 was able to bind CD16 (both forms), while 2H7 scFv (SSS-S) H P238CH2 WCH3 was not able to bind CD16 (either form).

Figure 76 compares the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs to bind CD64 positive U937 cells. The results indicate that both constructs had high affinity for Fc γ RI, and that the P238S mutation selectively reduced binding to Fc γ RIII.

Figure 77 illustrates an *in vivo* experiment in macaques to measure the effect of where 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs on B cell depletion. The constructs were administered one week apart and B cells were measures on days -7, 0, 1, 3, 7, 8, 10, 14, 28 and 43 using complete blood counts and two-color flow cytometry. The results indicate that the 2H7 scFv (SSS-S) H WCH2 WCH3 construct resulted in rapid and complete depletion of B cells, which lasted up to 28 days after the second injection. Conversely the 2H7 scFv (SSS-S) H P238CH2

WCH3 construct resulted in a slow reduction in B cells to about 50% in the first 2 weeks; however, B cells rapidly began to return to regular level shortly after this. This suggests that ADCC mediated by CD16 interaction is likely necessary for rapid and sustained B cell depletion.

5 **Figure 78** illustrates the SEC profiles of G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 constructs. The construct with an SSS hinge generated a single uniform peak at approximately 75-100 Kd, while the construct with a SSC hinge generated a smaller form and other heterogeneous forms, including one at greater than 200 Kd.

10 **Figure 79** illustrates the binding ability of G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 constructs in B cell lymphoma cells: Bjab, Ramos, WIL-2, Namalwa and Raji. The results in (a) indicate that G28-1 VL11S scFv (SSS) H WCH2 WCH3 bound to Bjab and Ramos cells, and moderately to WIL-2 cells, and at lower levels to Namalwa and Raji cells. The results in
15 (b) indicate the G28-1 VL11S scFv (SSC) H WCH2 WCH3 bound to Bjab cells, and moderately to and Ramos and WIL-2 cells, and at lower levels to Namalwa and Raji cells.

Figure 80 illustrates annexin and v-propidium iodide binding in Ramos cells incubated with G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 constructs overnight. The results indicate that both constructs
20 induced apoptosis; however, the construct with the (SSC) hinge induced more apoptosis than the construct with the (SSS) hinge.

Figure 81 illustrates the ability of G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 constructs to inhibit proliferation of Ramos cells. The results indicate that both constructs inhibited proliferation.

25 **Figure 82** illustrates the ability of G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 and 2H7 scFv (CSS) H WCH2 WCH3 constructs to induce apoptosis in Ramos B cells, alone and in different combinations. The results shown in this experiment indicate that the both G28-1 constructs were more efficient than the 2H7 construct. The results also indicate that the G28-1
30 VL11S scFv (SSS) H WCH2 WCH3 construct was more efficient than the G28-1 VL11S scFv (SSC) H WCH2 WCH3 construct. However, the amount of apoptosis was greatest when the 2H7 and the G28-1 constructs were used in combination.

Figure 83 compares the ability of G28-1 VL11S scFv (SSS) H WCH2 WCH3, G28-1 VL11S scFv (SSC) H WCH2 WCH3 and 2H7 scFv (CSS) H WCH2 WCH3 constructs to mediate CDC in Ramos B cells. The results indicate that all the constructs mediated CDC. The 2H7 construct was the most efficient, followed by G28-1 VL11S scFv (SSC) H WCH2 WCH3, then G28-1 VL11S scFv (SSS) H WCH2 WCH3.

Figure 84 compares the ability of the G28-1 VL11S scFv (SSS) H WCH2 WCH3, G28-1 VL11S scFv (SSC) H WCH2 WCH3 and 2H7 scFv (CSS) H WCH2 WCH3 constructs to mediate ADCC in Ramos B cells. The results indicate that the G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 constructs were able to mediate ADCC, while the 2H7 scFv (CSS) H WCH2 WCH3 construct ability to mediate ADCC was lower, but still higher than the level of natural killing.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to novel molecules useful, for example, as therapeutics, as well as for other purposes including diagnostic and research purposes. Such molecules have, for example, antigen binding or other binding function(s) and, for example, one or more effector functions. The invention includes molecular constructs, including binding domain-immunoglobulin fusion proteins, and related compositions and methods, which will be useful in immunotherapeutic and immunodiagnostic applications, and in research methods, and which offer certain advantages over antigen-specific compounds and polypeptides of the prior art. The constructs, including fusion proteins, of the present invention are preferably single polypeptide chains that comprise, in pertinent part, the following fused or otherwise connected domains or regions: a binding region construct, such as a binding domain or polypeptide, a connecting region construct including, for example, a native or engineered immunoglobulin hinge region polypeptide, and a tail region construct, including, for example, a construct that may comprise, consist essentially of, or consist of, a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide. According to certain embodiments that are particularly useful for gene therapy, the constructs, including fusion proteins, of the present invention may further comprise a native or engineered plasma membrane anchor domain. According to certain other preferred embodiments the constructs, including fusion proteins, of the present invention may further include a tail region having a native or engineered

immunoglobulin heavy chain CH4 constant region polypeptide. In particularly preferred embodiments, the binding regions, such as polypeptide domains, of which the constructs, including binding domain-immunoglobulin fusion proteins, are comprised are, or are derived from, polypeptides that are the products of human gene sequences, but the invention need not be so limited and may in fact relate to constructs, including binding domain-immunoglobulin fusion proteins, as provided herein that are derived from any natural or artificial source, including genetically engineered and/or mutated polypeptides.

The present invention relates in part to the surprising observation that the novel constructs, including binding domain-immunoglobulin fusion proteins, described herein are capable of immunological activity. More specifically, these proteins retain the ability to participate in well known immunological effector activities including, for example, antibody dependent cell mediated cytotoxicity (*e.g.*, subsequent to antigen binding on a cell surface, engagement and induction of cytotoxic effector cells bearing appropriate Fc receptors, such as Natural Killer cells bearing Fc γ III, under appropriate conditions) and/or complement fixation in complement dependent cytotoxicity (*e.g.*, subsequent to antigen binding on a cell surface, recruitment and activation of cytolytic proteins that are components of the blood complement cascade) despite having structures not be expected to be capable of promoting such effector activities or to promotion of such activities as described herein. For reviews of ADCC and CDC see, *e.g.*, Carter, 2001 *Nat. Rev. Canc.* 1:118; Sulica *et al.*, 2001 *Int. Rev. Immunol.* 20:371; Maloney *et al.*, 2002 *Semin. Oncol.* 29:2; Sondel *et al.*, 2001 *Hematol Oncol Clin North Am* 15(4):703-21; Maloney 2001 *Anticanc. Drugs* 12 Suppl.2:1-4. IgA activation of complement by the alternative pathway is described, for example, in Schneiderman *et al.*, 1990 *J. Immunol.* 145:233.. As described in greater detail below, ADCC, complement fixation, and CDC are unexpected functions for constructs, including fusion proteins, comprising for example immunoglobulin heavy chain regions and having the structures described herein, and in particular for immunoglobulin fusion proteins comprising, for example, immunoglobulin hinge region polypeptides that are compromised in their ability to form interchain, homodimeric disulfide bonds.

Another advantage afforded by the present invention is constructs, including binding domain-immunoglobulin fusion polypeptides, of the invention that can be produced in substantial quantities that are typically greater than those routinely attained

with single-chain antibody constructs of the prior art, for example. In preferred embodiments, constructs, including the binding domain-immunoglobulin fusion polypeptides, of the present invention are recombinantly expressed in mammalian or other desired and useful expression systems, which offer the advantage of providing polypeptides that are stable *in vivo* (e.g., under physiological conditions). According to non-limiting theory, such stability may derive in part from posttranslational modifications, and specifically glycosylation. Production of the constructs, including binding domain-immunoglobulin fusion protein constructs, of the invention via recombinant mammalian expression has been attained in static cell cultures at a level of greater than 50 mg protein per liter culture supernatant and has been routinely observed in such cultures at 10-50 mg/liter, such that preferably at least 10-50 mg/liter may be produced under static culture conditions; also contemplated are enhanced production, in whole or in part, of the protein constructs of the invention using art-accepted scale-up methodologies such as "fed batch" (*i.e.*, non-static) production, where yields of at least 5-500 mg/l, and in some instances at least 0.5-1 gm/l, depending on the particular protein product, are obtained.

A construct, including a binding domain polypeptide, according to the present invention may be, for example, any polypeptide that possesses the ability to specifically recognize and bind to a cognate biological molecule or complex of more than one molecule or assembly or aggregate, whether stable or transient, of such a molecule. Such molecules include proteins, polypeptides, peptides, amino acids, or derivatives thereof; lipids, fatty acids or the like, or derivatives thereof; carbohydrates, saccharides or the like or derivatives thereof; nucleic acids, nucleotides, nucleosides, purines, pyrimidines or related molecules, or derivatives thereof, or the like; or any combination thereof such as, for example, glycoproteins, glycopeptides, glycolipids, lipoproteins, proteolipids; or any other biological molecule that may be present in a biological sample. Biological samples may be provided, for example, by obtaining a blood sample, biopsy specimen, tissue explant, organ culture, biological fluid or any other tissue or cell or other preparation from a subject or a biological source. The subject or biological source may, for example, be a human or non-human animal, a primary cell culture or culture adapted cell line including but not limited to genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid cell lines, differentiated or differentiable

cell lines, transformed cell lines and the like, *etc.* In certain preferred embodiments of the invention, the subject or biological source may be suspected of having or being at risk for having a disease, disorder or condition, including a malignant disease, disorder or condition or a B cell disorder, which in certain further embodiments may be an autoimmune disease, and in certain other embodiments of the invention the subject or biological source may be known to be free of a risk or presence of such disease, disorder or condition.

A binding region, including a binding domain polypeptide, for example, may be any naturally occurring, synthetic, semi-synthetic, and/or recombinantly produced binding partner for a biological or other molecule that is a target structure of interest, herein sometimes referred to as an "antigen" but intended according to the present disclosure to encompass any target biological or other molecule to which it is desirable to have the subject invention, for example, a fusion protein, bind or specifically bind. Constructs of the invention, including binding domain-immunoglobulin fusion proteins, are defined to be "immunospecific" or capable of binding to a desired degree, including specifically binding, if they bind a desired target molecule such as an antigen as provided herein, at a desired level, for example, with a K_a of greater than or equal to about 10^4 M^{-1} , preferably of greater than or equal to about 10^5 M^{-1} , more preferably of greater than or equal to about 10^6 M^{-1} and still more preferably of greater than or equal to about 10^7 M^{-1} . Affinities of even greater than about 10^7 M^{-1} are still more preferred, such as affinities equal to or greater than about 10^7 M^{-1} , about 10^8 M^{-1} , and about 10^9 M^{-1} , and about 10^{10} M^{-1} . Affinities of binding domain-immunoglobulin fusion proteins according to the present invention can be readily determined using conventional techniques, for example those described by Scatchard *et al.*, 1949 *Ann. N.Y. Acad. Sci.* 51:660. Such determination of fusion protein binding to target antigens of interest can also be performed using any of a number of known methods for identifying and obtaining proteins that specifically interact with other proteins or polypeptides, for example, a yeast two-hybrid screening system such as that described in U.S. Patent No. 5,283,173 and U.S. Patent No. 5,468,614, or the equivalent.

Preferred embodiments of the subject invention constructs, for example, binding domain-immunoglobulin fusion proteins, comprise binding regions or binding domains that may include, for example, at least one native or engineered immunoglobulin

variable region polypeptide, such as all or a portion or fragment of a native or engineered heavy chain and/or a native or engineered light chain V-region, provided it is capable of binding or specifically binding an antigen or other desired target structure of interest at a desired level of binding and selectivity. In other preferred embodiments the binding region or binding domain comprises, consists essentially of, or consists of, a single chain immunoglobulin-derived Fv product, for example, and scFv, which may include all or a portion of at least one native or engineered immunoglobulin light chain V-region and all or a portion of at least one native or engineered immunoglobulin heavy chain V-region, and a linker fused or otherwise connected to the V-regions; preparation and testing such constructs are described in greater detail herein. Other preparation and testing methods are well known in the art.

As described herein and known in the art, immunoglobulins comprise products of a gene family the members of which exhibit a high degree of sequence conservation. Amino acid sequences of two or more immunoglobulins or immunoglobulin domains or regions or portions thereof (*e.g.*, V_H domains, V_L domains, hinge regions, CH2 constant regions, CH3 constant regions) may be aligned and analyzed. Portions of sequences that correspond to one another may be identified, for instance, by sequence homology. Determination of sequence homology may be determined with any of a number of sequence alignment and analysis tools, including computer algorithms well known to those of ordinary skill in the art, such as Align or the BLAST algorithm (Altschul, 1991 *J. Mol. Biol.* **219**:555-565; Henikoff and Henikoff, 1992 *Proc. Natl. Acad. Sci. USA* **89**:10915-10919), which is available at the NCBI website (<http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST>). Default parameters may be used.

Portions, for example, of a particular immunoglobulin reference sequence and of any one or more additional immunoglobulin sequences of interest that may be compared to a reference sequence. "Corresponding" sequences, regions, fragments or the like, may be identified based on the convention for numbering immunoglobulin amino acid positions according to Kabat, *Sequences of Proteins of Immunological Interest*, (5th ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)). For example, according to this convention, the immunoglobulin family to which an immunoglobulin sequence of interest belongs is determined based on conservation of variable region polypeptide sequence invariant amino acid residues, to identify a particular numbering

system for the immunoglobulin family, and the sequence(s) of interest can then be aligned to assign sequence position numbers to the individual amino acids which comprise such sequence(s). Preferably at least about 70%, more preferably at least about 80%-85% or about 86%-89%, and still more preferably at least about 90%, about 92%, about 94%, about 96%, about 98% or about 99% of the amino acids in a given amino acid sequence of at least about 1000, more preferably about 700-950, more preferably about 350-700, still more preferably about 100-350, still more preferably about 80-100, about 70-80, about 60-70, about 50-60, about 40-50 or about 30-40 consecutive amino acids of a sequence, are identical to the amino acids located at corresponding positions in a reference sequence such as those disclosed by Kabat (1991) or in a similar compendium of related immunoglobulin sequences, such as may be generated from public databases (*e.g.*, Genbank, SwissProt, *etc.*) using sequence alignment tools such as, for example, those described above. In certain preferred embodiments, an immunoglobulin sequence of interest or a region, portion, derivative or fragment thereof is greater than about 95% identical to a corresponding reference sequence, and in certain preferred embodiments such a sequence of interest may differ from a corresponding reference at no more than about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid positions:

For example, in certain embodiments the present invention is directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in pertinent part a human or other species immunoglobulin heavy chain variable region polypeptide comprising a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112 in, for example, SEQ ID NO: __, which comprises, for example, a murine V_H-derived sequence. At a relatively limited number of immunoglobulin V_H sequence positions, for example, including position 11, amino acid conservation is observed in the overwhelming majority of V_H sequences analyzed across mammalian species lines (*e.g.*, Leu11, Val37, Gly44, Leu45, Trp47; Nguyen *et al.*, 1998 *J. Mol. Biol.* 275:413). Various such amino acid residues, and hence their side chains, are located at the surface of the variable domain (V_H). They may contact residues of the C_H1 (*e.g.*, Leu11) and the V_L domains (*e.g.*, Val37, Gly44, Leu45, and Trp47) and may, in the absence of light chains, contribute to stability and solubility of the protein (*see, e.g.*, Chothia *et al.*, 1985 *J. Mol. Biol.* 186:651; Muyldermans *et al.*, 1994 *Prot. Engineer.* 7:1129; Desmyter *et al.*, 1996

Nat. Struct. Biol. 3:803; Davies *et al.*, 1994 *FEBS Lett.* 339:285). In certain embodiments, for example, the present invention is also directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in pertinent part a human immunoglobulin light chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108. In still other certain embodiments, for example, the present invention is directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in pertinent part (1) a human immunoglobulin heavy chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising, consisting essentially of, or consisting of, said heavy chain sequence having a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, and (2) a human immunoglobulin light chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising, consisting essentially of, or consisting of, said light chain sequence having a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108.

As another example, by reference to immunoglobulin sequence compendia and databases such as those cited above, for example, the relatedness of two or more immunoglobulin sequences to each other can readily and without undue experimentation be established in a manner that permits identification of the animal species of origin, the class and subclass (*e.g.*, isotype) of a particular immunoglobulin or immunoglobulin region polypeptide sequence. Any immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences or other native or engineered V region-derived sequences or the like, may be used as a binding region or binding domain. Engineered sequences includes immunoglobulin sequences from any species, preferably human or mouse, for example, that include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112 in a heavy chain variable region sequence or an scFv, and/or a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid

positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence or an scFv.

Various preferred embodiments include, for example, native or engineered immunoglobulin V region polypeptide sequences derived, for example, from antibodies including monoclonal antibodies such as murine or other rodent antibodies, or antibodies or monoclonal antibodies derived from other sources such as goat, rabbit, equine, bovine, camelid or other species, including transgenic animals, and also including human or humanized antibodies or monoclonal antibodies. Non-limiting examples include variable region polypeptide sequences derived from monoclonal antibodies such as those referenced herein and/or described in greater detail in the Examples below, for instance, CD20-binding or specific murine monoclonal antibodies (*e.g.*, 2H7), other CD20-binding or specific murine monoclonal antibodies that are not 1F5 antibodies, monoclonal antibody L6 (specific for a carbohydrate-defined epitope and available from American Type Culture Collection, Manassas, VA, as hybridoma HB8677), and monoclonal antibodies that bind to or are specific for CD28 (*e.g.*, monoclonal antibody 2E12), CD40, CD80, CD137 (*e.g.*, monoclonal antibody 5B9 or monoclonal antibody 1D8 which recognizes the murine homologue of CD137, 41BB) and CD152 (CTLA-4).

Other binding regions, including binding domain polypeptides, may comprise any protein or portion thereof that retains the ability to bind or specifically bind to an antigen as provided herein, including non-immunoglobulins. Accordingly the invention contemplates constructs, including fusion proteins, comprising binding region or binding domain polypeptides that are derived from polypeptide ligands such as hormones, cytokines, chemokines, and the like; cell surface or soluble receptors for such polypeptide ligands; lectins; intercellular adhesion receptors such as specific leukocyte integrins, selectins, immunoglobulin gene superfamily members, intercellular adhesion molecules (ICAM-1, -2, -3) and the like; histocompatibility antigens; *etc.*

Examples of cell surface receptors useful in the preparation of, or as, binding regions, or that may provide a binding domain polypeptide, and that may also be selected as a target molecule or antigen to which a construct, including for example, a binding domain-Ig fusion protein of the present invention desirably binds, include the following, or the like: HER1 (*e.g.*, GenBank Accession Nos. U48722, SEG_HEGFREXS, KO3193), HER2 (Yoshino *et al.*, 1994 *J. Immunol.* 152:2393; Disis *et al.*, 1994 *Canc. Res.*

54:16; see also, *e.g.*, GenBank Acc. Nos. X03363, M17730, SEG_HUMHER20), HER3 (*e.g.*, GenBank Acc. Nos. U29339, M34309), HER4 (Plowman *et al.*, 1993 *Nature* 366:473; see also *e.g.*, GenBank Acc. Nos. L07868, T64105), epidermal growth factor receptor (EGFR) (*e.g.*, GenBank Acc. Nos. U48722, SEG_HEGFREXS, KO3193),
5 vascular endothelial cell growth factor (*e.g.*, GenBank No. M32977), vascular endothelial cell growth factor receptor (VEGF) (*e.g.*, GenBank Acc. Nos. AF022375, 1680143, U48801, X62568), insulin-like growth factor-I (*e.g.*, GenBank Acc. Nos. X00173, X56774, X56773, X06043, *see also* European Patent No. GB 2241703), insulin-like growth factor-II (*e.g.*, GenBank Acc. Nos. X03562, X00910, SEG_HUMGFIA, SEG_HUMGFII, M17863,
10 M17862), transferrin receptor (Trowbridge and Omary, 1981 *Proc. Nat. Acad. USA* 78:3039; see also *e.g.*, GenBank Acc. Nos. X01060, M11507), estrogen receptor (*e.g.*, GenBank Acc. Nos. M38651, X03635, X99101, U47678, M12674), progesterone receptor (*e.g.*, GenBank Acc. Nos. X51730, X69068, M15716), follicle stimulating hormone receptor (FSH-R) (*e.g.*, GenBank Acc. Nos. Z34260, M65085), retinoic acid receptor (*e.g.*,
15 GenBank Acc. Nos. L12060, M60909, X77664, X57280, X07282, X06538), MUC-1 (Barnes *et al.*, 1989 *Proc. Nat. Acad. Sci. USA* 86:7159; see also *e.g.*, GenBank Acc. Nos. SEG_MUSMUCIO, M65132, M64928) NY-ESO-1 (*e.g.*, GenBank Acc. Nos. AJ003149, U87459), NA 17-A (*e.g.*, European Patent No. WO 96/40039), Melan-A/MART-1 (Kawakami *et al.*, 1994 *Proc. Nat. Acad. Sci. USA* 91:3515; see also *e.g.*, GenBank Acc.
20 Nos. U06654, U06452), tyrosinase (Topalian *et al.*, 1994 *Proc. Nat. Acad. Sci. USA* 91:9461; see also *e.g.*, GenBank Acc. Nos. M26729, SEG_HUMTYR0, *see also* Weber *et al.*, *J. Clin. Invest* (1998) 102:1258), Gp-100 (Kawakami *et al.*, 1994 *Proc. Nat. Acad. Sci. USA* 91:3515; see also *e.g.*, GenBank Acc. No. S73003, *see also* European Patent No. EP 668350; Adema *et al.*, 1994 *J. Biol. Chem.* 269:20126), MAGE (van den Bruggen *et al.*,
25 1991 *Science* 254:1643; see also *e.g.*, GenBank Acc. Nos. U93163, AF064589, U66083, D32077, D32076, D32075, U10694, U10693, U10691, U10690, U10689, U10688, U10687, U10686, U10685, L18877, U10340, U10339, L18920, U03735, M77481), BAGE (*e.g.*, GenBank Acc. No. U19180; see also U.S. Patent Nos. 5,683,886 and 5,571,711), GAGE (*e.g.*, GenBank Acc. Nos. AF055475, AF055474, AF055473, U19147, U19146,
30 U19145, U19144, U19143, U19142), any of the CTA class of receptors including in particular HOM-MEL-40 antigen encoded by the SSX2 gene (*e.g.*, GenBank Acc. Nos. X86175, U90842, U90841, X86174), carcinoembryonic antigen (CEA, Gold and Freedman,

1985 *J. Exp. Med.* **121**:439; see also e.g., GenBank Acc. Nos. SEG_HUMCEA, M59710, M59255, M29540), and PyLT (e.g., GenBank Acc. Nos. J02289, J02038).

Additional cell surface receptors that may be sources of binding region or binding domain polypeptides or portions thereof, or that may be targets, including target
5 antigens, include the following, or the like: CD2 (e.g., GenBank Acc. Nos. Y00023, SEG_HUMCD2, M16336, M16445, SEG_MUSCD2, M14362), 4-1BB (CDw137, Kwon
et al., 1989 *Proc. Nat. Acad. Sci. USA* **86**:1963, 4-1BB ligand (Goodwin *et al.*, 1993 *Eur.*
J. Immunol. **23**:2361; Melero *et al.*, 1998 *Eur. J. Immunol.* **3**:116), CD5 (e.g., GenBank
Acc. Nos. X78985, X89405), CD10 (e.g., GenBank Acc. Nos. M81591, X76732) CD27
10 (e.g., GenBank Acc. Nos. M63928, L24495, L08096), CD28 (June *et al.*, 1990 *Immunol.*
Today **11**:211; see also, e.g., GenBank Acc. Nos. J02988, SEG_HUMCD28, M34563),
CD152/CTLA-4 (e.g., GenBank Acc. Nos. L15006, X05719, SEG_HUMIGCTL), CD40
(e.g., GenBank Acc. Nos. M83312, SEG_MUSC040A0, Y10507, X67878, X96710,
U15637, L07414), interferon- γ (IFN- γ ; see, e.g., Farrar *et al.* 1993 *Ann. Rev. Immunol.*
15 **11**:571 and references cited therein, Gray *et al.* 1982 *Nature* **295**:503, Rinderknecht *et al.*
1984 *J. Biol. Chem.* **259**:6790, DeGrado *et al.* 1982 *Nature* **300**:379), interleukin-4 (IL-4;
see, e.g., 53rd *Forum in Immunology*, 1993 *Research in Immunol.* **144**:553-643;
Banchereau *et al.*, 1994 in *The Cytokine Handbook*, 2nd ed., A. Thomson, ed., Academic
Press, NY, p. 99; Keegan *et al.*, 1994 *J. Leukocyt. Biol.* **55**:272, and references cited
20 therein), interleukin-17 (IL-17) (e.g., GenBank Acc. Nos. U32659, U43088) and
interleukin-17 receptor (IL-17R) (e.g., GenBank Acc. Nos. U31993, U58917).
Notwithstanding the foregoing, the present invention expressly does not encompass any
immunoglobulin fusion protein that is disclosed in U.S. 5,807,734, or U.S. 5,795,572.

Additional cell surface receptors that may be sources of binding region or
25 binding domain polypeptides or portions thereof, or that may serve as targets including
target antigens or binding sites include the following, or the like: CD59 (e.g., GenBank
Acc. Nos. SEG_HUMCD590, M95708, M34671), CD48 (e.g., GenBank Acc. Nos.
M59904), CD58/LFA-3 (e.g., GenBank Acc. No. A25933, Y00636, E12817; see also JP
1997075090-A), CD72 (e.g., GenBank Acc. Nos. AA311036, S40777, L35772), CD70
30 (e.g., GenBank Acc. Nos. Y13636, S69339), CD80/B7.1 (Freeman *et al.*, 1989 *J. Immunol.*
43:2714; Freeman *et al.*, 1991 *J. Exp. Med.* **174**:625; see also e.g., GenBank Acc. Nos.
U33208, I683379), CD86/B7.2 (Freeman *et al.*, 1993 *J. Exp. Med.* **178**:2185, Boriello *et*

al., 1995 *J. Immunol.* **155**:5490; see also, *e.g.*, GenBank Acc. Nos. AF099105, SEG_MMB72G, U39466, U04343, SEG_HSB725, L25606, L25259), B7-H1/B7-DC (*e.g.*, Genbank Acc. Nos. NM_014143, AF177937, AF317088; Dong *et al.*, 2002 *Nat. Med.* Jun 24 [epub ahead of print], PMID 12091876; Tseng *et al.*, 2001 *J. Exp. Med.* **193**:839;

5 Tamura *et al.*, 2001 *Blood* **97**:1809; Dong *et al.*, 1999 *Nat. Med.* **5**:1365), CD40 ligand (*e.g.*, GenBank Acc. Nos. SEG_HUMCD40L, X67878, X65453, L07414), IL-17 (*e.g.*, GenBank Acc. Nos. U32659, U43088), CD43 (*e.g.*, GenBank Acc. Nos. X52075, J04536), ICOS (*e.g.*, Genbank Acc. No. AH011568), CD3 (*e.g.*, Genbank Acc. Nos. NM_000073 (gamma subunit), NM_000733 (epsilon subunit), X73617 (delta subunit)), CD4 (*e.g.*,

10 Genbank Acc. No. NM_000616), CD25 (*e.g.*, Genbank Acc. No. NM_000417), CD8 (*e.g.*, Genbank Acc. No. M12828), CD11b (*e.g.*, Genbank Acc. No. J03925), CD14 (*e.g.*, Genbank Acc. No. XM_039364), CD56 (*e.g.*, Genbank Acc. No. U63041), CD69 (*e.g.*, Genbank Acc. No. NM_001781) and VLA-4 ($\alpha_4\beta_7$) (*e.g.*, GenBank Acc. Nos. L12002, X16983, L20788, U97031, L24913, M68892, M95632). The following cell surface

15 receptors are typically associated with B cells: CD19 (*e.g.*, GenBank Acc. Nos. SEG_HUMCD19W0, M84371, SEG_MUSCD19W, M62542), CD20 (*e.g.*, GenBank Acc. Nos. SEG_HUMCD20, M62541), CD22 (*e.g.*, GenBank Acc. Nos. I680629, Y10210, X59350, U62631, X52782, L16928), CD30 (*e.g.*, Genbank Acc. Nos. M83554, D86042), CD153 (CD30 ligand, *e.g.*, GenBank Acc. Nos. L09753, M83554), CD37 (*e.g.*, GenBank

20 Acc. Nos. SEG_MMCD37X, X14046, X53517), CD50 (ICAM-3, *e.g.*, GenBank Acc. No. NM_002162), CD106 (VCAM-1) (*e.g.*, GenBank Acc. Nos. X53051, X67783, SEG_MMVCAM1C, *see also* U.S. Patent No. 5,596,090), CD54 (ICAM-1) (*e.g.*, GenBank Acc. Nos. X84737, S82847, X06990, J03132, SEG_MUSICAM0), interleukin-12 (see, *e.g.*, Reiter *et al.*, 1993 *Crit. Rev. Immunol.* **13**:1, and references cited therein),

25 CD134 (OX40, *e.g.*, GenBank Acc. No. AJ277151), CD137 (41BB, *e.g.*, GenBank Acc. No. L12964, NM_001561), CD83 (*e.g.*, GenBank Acc. Nos. AF001036, AL021918), DEC-205 (*e.g.*, GenBank Acc. Nos. AF011333, U19271).

Constructs, including binding domain-immunoglobulin fusion proteins, of the present invention comprise, for example, a binding domain, such as a binding domain

30 polypeptide that, according to certain particularly preferred embodiments, is capable of binding or specifically binding at least one target, for example, a target antigen or other binding site that is present on an immune effector cell. According to non-limiting theory,

such constructs, including for example binding domain- immunoglobulin fusion proteins, may advantageously recruit desired immune effector cell function(s) in a therapeutic context, where it is well known that immune effector cells having different specialized immune functions can be identified or distinguished from one another on the basis of their differential expression of a wide variety of cell surface antigens, including many of the antigens described herein to which constructs of the invention including binding domain polypeptides can specifically bind. As noted herein, immune effector cells include any cell that is capable of directly mediating an activity that is a component of immune system function, including cells having such capability naturally or as a result of genetic engineering.

In certain embodiments an immune effector cell comprises a cell surface receptor for an immunoglobulin or other peptide binding molecule, such as a receptor for an immunoglobulin constant region and including the class of receptors commonly referred to as "Fc receptors" ("FcR"s). A number of FcRs have been structurally and/or functionally characterized and are well known in the art, including FcR having specific abilities to interact with a restricted subset of immunoglobulin heavy chain isotypes, or that interact with Fc domains with varying affinities, and/or which may be expressed on restricted subsets of immune effector cells under certain conditions (*e.g.*, Kijimoto-Ochichai *et al.*, 2002 *Cell Mol. Life Sci.* **59**:648; Davis *et al.*, 2002 *Curr. Top. Microbiol. Immunol.* **266**:85; Pawankar, 2001 *Curr. Opin. Allerg. Clin. Immunol.* **1**:3; Radaev *et al.*, 2002 *Mol. Immunol.* **38**:1073; Wurzburg *et al.*, 2002 *Mol. Immunol.* **38**:1063; Sulica *et al.*, 2001 *Int. Rev. Immunol.* **20**:371; Underhill *et al.*, 2002 *Ann. Rev. Immunol.* **20**:825; Coggeshall, 2002 *Curr. Dir. Autoimm.* **5**:1; Mimura *et al.*, 2001 *Adv. Exp. Med. Biol.* **495**:49; Baumann *et al.*, 2001 *Adv. Exp. Med. Biol.* **495**:219; Santoso *et al.*, 2001 *Ital. Heart J.* **2**:811; Novak *et al.*, 2001 *Curr. Opin. Immunol.* **13**:721; Fossati *et al.*, 2001 *Eur. J. Clin. Invest.* **31**:821).

Cells that are capable of mediating ADCC are preferred examples of immune effector cells according to the present invention. Other preferred examples include Natural Killer cells, tumor-infiltrating T lymphocytes (TILs), cytotoxic T lymphocytes, and granulocytic cells such as cells that comprise allergic response mechanisms. Immune effector cells thus include, but are not limited to, cells of hematopoietic origins including cells at various stages of differentiation within myeloid

and lymphoid lineages and which may (but need not) express one or more types of functional cell surface FcR, such as T lymphocytes, B lymphocytes, NK cells, monocytes, macrophages, dendritic cells, neutrophils, basophils, eosinophils, mast cells, platelets, erythrocytes, and precursors, progenitors (*e.g.*, hematopoietic stem cells), as well as quiescent, activated, and mature forms of such cells. Other immune effector cells may include cells of non-hematopoietic origin that are capable of mediating immune functions, for example, endothelial cells, keratinocytes, fibroblasts, osteoclasts, epithelial cells, and other cells. Immune effector cells may also include cells that mediate cytotoxic or cytostatic events, or endocytic, phagocytic, or pinocytotic events, or that effect induction of apoptosis, or that effect microbial immunity or neutralization of microbial infection, or cells that mediate allergic, inflammatory, hypersensitivity and/or autoimmune reactions.

Allergic response mechanisms are well known in the art and include an antigen (*e.g.*, allergen)-specific component such as an immunoglobulin (*e.g.*, IgE), as well as the cells and mediators which comprise sequelae to allergen-immunoglobulin (*e.g.*, IgE) encounters (*e.g.*, Ott *et al.*, 2000 *J. Allerg. Clin. Immunol.* 106:429; Barnes, 2000 *J. Allerg. Clin. Immunol.* 106:5; Togias, 2000 *J. Allerg. Clin. Immunol.* 105:S599; Akdis *et al.*, 2000 *Int. Arch. Allerg. Immunol.* 121:261; Beach, 2000 *Occup. Med.* 15:455). Particularly with regard to constructs, including binding domain-immunoglobulin fusion proteins, of the present invention that interact with FcR, certain embodiments of the present invention contemplate constructs including fusion proteins that comprise one or more IgE-derived domains including, for example, those that are capable of inducing an allergic response mechanism that comprises IgE-specific FcR, or portions thereof, which IgE-specific FcRs include those noted above and described or identified in the cited articles. Without wishing to be bound by particular theory or mechanism, and as disclosed herein, constructs, including fusion proteins, of the present invention may comprise portions of IgE heavy chain Fc domain polypeptides, for example, native or engineered IgE CH3 and CH4 domains, whether provided or expressed as cell surface proteins (*e.g.*, with a plasma membrane anchor domain) or as soluble or otherwise not cell-bound proteins (*e.g.*, without a plasma membrane anchor domain). Further according to non-limiting theory, recruitment and induction of an allergic response mechanism (*e.g.*, an FcR-epsilon expressing immune effector cell) may proceed as the result of either or both of the presence of an IgE Fc domain or portion thereof as described herein (*e.g.*, one that is capable of triggering an

allergic mechanism by FcR crosslinking) and the presence of a target such as an antigen to which the binding region, for example a binding domain, binds or specifically binds. The present invention therefore exploits induction of allergic response mechanisms in heretofore unappreciated contexts, such as treatment of a malignant condition or a B cell disorder, including those described or referenced herein.

An immunoglobulin hinge region polypeptide includes any hinge peptide or polypeptide that occurs naturally, as an artificial peptide or as the result of genetic engineering and that is situated, for example, in an immunoglobulin heavy chain polypeptide between the amino acid residues responsible for forming intrachain immunoglobulin-domain disulfide bonds in CH1 and CH2 regions. Hinge region polypeptides for use in the present invention may also include a mutated or otherwise altered hinge region polypeptide. Accordingly, for example, an immunoglobulin hinge region polypeptide may be derived from, or may be a portion or fragment of (*i.e.*, one or more amino acids in peptide linkage, typically about 15-115 amino acids, preferably about 95-110, about 80-94, about 60-80, or about 5-65 amino acids, preferably about 10-50, more preferably about 15-35, still more preferably about 18-32, still more preferably about 20-30, still more preferably about 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acids) an immunoglobulin polypeptide chain region classically regarded as having hinge function, including those described herein, but a hinge region polypeptide for use in the instant invention need not be so restricted and may include one or more amino acids situated (according to structural criteria for assigning a particular residue to a particular domain that may vary, as known in the art) in an adjoining immunoglobulin domain such as a CH1 domain and/or a CH2 domain in the cases of IgG, IgA and IgD (or in an adjoining immunoglobulin domain such as a CH1 domain and/or a CH3 domain in the case of IgE), or in the case of certain artificially engineered immunoglobulin constructs, an immunoglobulin variable region domain.

Wild-type immunoglobulin hinge region polypeptides include any known or later-discovered naturally occurring hinge region that is located between the constant region domains, CH1 and CH2, of an immunoglobulin, for example, a human immunoglobulin (or between the CH1 and CH3 regions of certain types of immunoglobulins, such as IgE). For use in constructing one type of connecting region, the wild-type immunoglobulin hinge region polypeptide is preferably a human

immunoglobulin hinge region polypeptide, preferably comprising a hinge region from a human IgG, IgA, or IgD immunoglobulin (or the CH2 region of an IgE immunoglobulin), and more preferably, for example, a hinge region polypeptide from a wild-type or mutated human IgG1 isotype as described herein.

5 As is known to the art, despite the tremendous overall diversity in immunoglobulin amino acid sequences, immunoglobulin primary structure exhibits a high degree of sequence conservation in particular portions of immunoglobulin polypeptide chains, notably with regard to the occurrence of cysteine residues which, by virtue of their
10 sulfhydryl groups, offer the potential for disulfide bond formation with other available sulfhydryl groups. Accordingly, in the context of the present invention wild-type immunoglobulin hinge region polypeptides for use as connecting regions include those that feature one or more highly conserved (*e.g.*, prevalent in a population in a statistically significant manner) cysteine residues, and in certain preferred embodiments a connecting region may comprise, or consist essentially of, or consist of, a mutated hinge region
15 polypeptide may be selected that contains less than the number of naturally-occurring cysteines, for example, zero or one or two cysteine residue(s) in the case of IgG1 and IgG4 hinge regions, and that is derived or constructed from (or using) such a wild-type hinge region sequence.

 In certain preferred embodiments wherein the connecting region is a hinge
20 region polypeptide and the hinge region polypeptide is a mutated, engineered or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that is derived or constructed from (or using) a wild-type hinge region sequence, it is noted that the wild-type human IgG1 hinge region polypeptide sequence comprises three non-adjacent cysteine residues, referred to as a first cysteine of the wild-type hinge region, a second cysteine of
25 the wild-type hinge region and a third cysteine of the wild-type hinge region, respectively, proceeding along the hinge region sequence from the polypeptide N-terminus toward the C-terminus. This can be referred to herein as a "CCC" hinge (or a "WTH", *i.e.*, a wild-type hinge). Examples of mutated or engineered hinge regions include those with no cysteines, which may be referred to herein as an "XXX" hinge (or, for example, as "MH-XXX,"
30 referring to a mutant or engineered hinge with three amino acids or other molecules in place of naturally occurring cysteines, such as, for example, "MH-SSS", which refers to a mutant hinge with three serine residues in place of the naturally occurring cysteine

residues. It will be understood that the term "mutant" refers only to the fact that a different molecule or molecules is present, or no molecule, at the position of a naturally occurring residue and does not refer to any particular method by which such substitution, alteration, or deletion has been carried out. Accordingly, in certain embodiments of the present invention, the connecting region may be a hinge region polypeptide and the hinge region polypeptide is a mutated human IgG1 immunoglobulin hinge region polypeptide that contains two cysteine residues and in which the first cysteine of the wild-type hinge region has not changed or deleted, for example. This can be referred to as a "MH-CXX" hinge, for example, a "MH-CSC" hinge, in which case the cysteine residue has been replaced with a serine residue. In certain other embodiments of the present invention the mutated human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue and include, for example, a "MH-CSS" hinge or a "MH-SSC" hinge or a "MH-CSC" hinge, and in certain other embodiments the mutated human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues such as, for example, a "MH-SSS" hinge.

The constructs, including binding domain-immunoglobulin fusion proteins, of the present invention expressly do not contemplate any fusion protein that is disclosed in U.S. Patent No. 5,892,019. U.S. Patent No. 5,892,019 refers to a human IgG1 hinge region in which the first IgG1 hinge region cysteine residue has been changed or deleted, but retains both of the second and third IgG1 hinge region cysteine residues that correspond to the second and third cysteines of the wild-type IgG1 hinge region sequence. The patent states that the first cysteine residue of the wild-type IgG1 hinge region is replaced to prevent interference by the first cysteine residue with proper assembly of the polypeptide described therein into a dimer. The patent requires that the second and third cysteines of the IgG1 hinge region be retained to provide interchain disulfide linkage between two heavy chain constant regions to promote dimer formation so that the molecule contains has effector function such as the ability to mediate ADCC.

By contrast and as described herein, the constructs, including the binding domain-immunoglobulin fusion proteins, of the present invention, various of which are capable of ADCC, CDC and/or complement fixation, for example, are not so limited and may comprise, in pertinent part, for example, (i) a wild-type immunoglobulin hinge region polypeptide, such as a wild-type human immunoglobulin hinge region polypeptide, for

example, a human IgG1 immunoglobulin hinge region polypeptide, (ii) a mutated or otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that, for example, is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated or deleted, (iii) a mutated or otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that, for example, is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue, or (iv) a mutated or otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered (for example, by amino acid change or deletion) human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues. The present invention offers unexpected advantages associated with retention by the constructs, including the fusion proteins, described herein of the ability to mediate ADCC and/or CDC and/or complement fixation notwithstanding that the ability to dimerize via IgG1 hinge region interchain disulfide bonds is ablated or compromised by the removal or replacement of one, two or three hinge region cysteine residues, and even in constructs where the first cysteine of an IgG1 hinge region, for example, is not mutated or otherwise altered or deleted.

A connecting region may comprise a mutated or otherwise altered immunoglobulin hinge region polypeptide, which itself may comprise a hinge region that

has its origin in an immunoglobulin of a species, of an immunoglobulin isotype or class, or of an immunoglobulin subclass that is different from that of the tail region, for example, a tail region comprising, or consisting essentially of, or consisting of, CH2 and CH3 domains (or IgE CH3 and CH4 domains). For instance, in certain embodiments of the invention, a
5 construct, for example, a binding domain-immunoglobulin fusion protein, may comprise a binding region such as a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide comprising, or consisting essentially of, or consisting of, a wild-type human IgA hinge region polypeptide, or a mutated or otherwise altered human IgA hinge region polypeptide that contains zero or only one or more
10 cysteine residues (but less than the wild-type number of cysteines), as described herein, or a wild-type human IgG hinge, such as an IgG1 hinge, region polypeptide, or a wild-type human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide, or a mutated or otherwise altered human IgG hinge, such as an IgG1 hinge, region polypeptide that is or has been mutated or otherwise altered to contain zero, one or two cysteine residues wherein
15 the first cysteine of the wild-type hinge region is not mutated or altered or deleted, as also described herein. Such a hinge region polypeptide may be fused or otherwise connected to, for example, a tail region comprising, or consisting essentially of, or consisting of, an immunoglobulin heavy chain CH2 region polypeptide from a different Ig isotype or class, for example an IgA or an IgD or an IgG subclass (or a CH3 region from an IgE subclass),
20 which in certain preferred embodiments will be the IgG1 or IgA or IgE subclass and in certain other preferred embodiments may be any one of the IgG2, IgG3 or IgG4 subclasses.

For example, and as described in greater detail herein, in certain embodiments of the present invention a connecting region may be selected to be an immunoglobulin hinge region polypeptide, which is or has been derived from a wild-type
25 human IgA hinge region that naturally comprises three cysteines, where the selected hinge region polypeptide is truncated or otherwise altered or substituted relative to the complete and/or naturally-occurring hinge region such that only one or two of the cysteine residues remain (*e.g.*, SEQ ID NOS:35-36). Similarly, in certain other embodiments of the invention, the construct may be binding domain-immunoglobulin fusion protein
30 comprising a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide comprising a mutated or otherwise altered hinge region polypeptide in which the number of cysteine residues is reduced by amino acid

substitution or deletion, for example a mutated or otherwise altered IgG1 hinge region containing zero, one or two cysteine residues as described herein, or an IgD hinge region containing zero cysteine residues.

5 A mutated or otherwise altered hinge region polypeptide may thus be derived or constructed from (or using) a wild-type immunoglobulin hinge region that contains one or more cysteine residues. In certain embodiments, a mutated or otherwise altered hinge region polypeptide may contain zero or only one cysteine residue, wherein the mutated or otherwise altered hinge region polypeptide is or has been derived from a wild type immunoglobulin hinge region that contains, respectively, one or more or two or
10 more cysteine residues. In the mutated or otherwise altered hinge region polypeptide, the cysteine residues of the wild-type immunoglobulin hinge region are preferably deleted or substituted with amino acids that are incapable of forming a disulfide bond. In one embodiment of the invention, a mutated or otherwise altered hinge region polypeptide is or has been derived from a human IgG wild-type hinge region polypeptide, which may
15 include any of the four human IgG isotype subclasses, IgG1, IgG2, IgG3 or IgG4. In certain preferred embodiments, the mutated or otherwise altered hinge region polypeptide is or has been derived from (or using) a human IgA or IgD wild-type hinge region polypeptide. By way of example, a mutated or otherwise altered hinge region polypeptide that is or has been derived from a human IgG1 or IgA wild-type hinge region polypeptide
20 may comprise mutations, alterations, or deletions at two of the three cysteine residues in the wild-type immunoglobulin hinge region, or mutations, alterations, or deletions at all three cysteine residues.

The cysteine residues that are present in a wild-type immunoglobulin hinge region and that are removed or altered by mutagenesis or any other techniques according to
25 particularly preferred embodiments of the present invention include cysteine residues that form, or that are capable of forming, interchain disulfide bonds. Without wishing to be bound by particular theory or mechanism of action, the present invention contemplates that mutation, deletion, or other alteration of such hinge region cysteine residues, which are believed to be involved in formation of interchain disulfide bridges, reduces the ability of
30 the subject invention binding domain-immunoglobulin fusion protein to dimerize (or form higher oligomers) via interchain disulfide bond formation, while surprisingly not ablating or undesireably compromising the ability of a fusion protein or other construct to promote

ADCC, and/or CDC and/or to fix complement. In particular, the Fc receptors that mediate ADCC (e.g., FcRIII, CD16) exhibit low affinity for immunoglobulin Fc domains, supporting the idea that functional binding of Fc to FcR requires avidity stabilization of the Fc-FcR complex by virtue of the dimeric structure of heavy chains in a conventional antibody, and/or FcR aggregation and cross-linking by a conventional antibody Fc structure. Sonderman *et al.*, 2000 *Nature* 406:267; Radaev *et al.*, 2001 *J. Biol. Chem.* 276:16469; Radaev *et al.*, 2001 *J. Biol. Chem.* 276:16478; Koolwijk *et al.*, 1989 *J. Immunol.* 143:1656; Kato *et al.*, 2000 *Immunol. Today* 21:310. Hence, the constructs, including for example binding domain-immunoglobulin fusion proteins, of the present invention provide the advantages associated with single-chain constructs including single-chain immunoglobulin fusion proteins while also unexpectedly retaining one or more immunological activities. Similarly, the ability to fix complement is typically associated with immunoglobulins that are dimeric with respect to heavy chain constant regions such as those that comprise Fc, while various constructs, including binding domain-immunoglobulin fusion proteins, of the present invention, which may, due to the replacement or deletion of hinge region cysteine residues or due to other structural modifications as described herein, for example, have compromised or ablated abilities to form interchain disulfide bonds, exhibit the unexpected ability to fix complement. Additionally, according to certain embodiments of the present invention wherein a construct, including, for example, a binding domain-immunoglobulin fusion protein, may comprise a connecting region and tail region comprising, or consisting essentially of, or consisting of, one or more of a human IgE hinge-acting region, *i.e.*, a IgE CH2 region polypeptide, a human IgE CH3 constant region polypeptide, and a human IgE CH4 constant region polypeptide, the invention constructs including fusion proteins unexpectedly retain the immunological activity of mediating ADCC and/or of inducing an allergic response mechanism.

Selection of an immunoglobulin hinge region polypeptide as a connecting region according to certain embodiments of the subject invention constructs, such as binding domain-immunoglobulin fusion proteins, may relate to the use of an "alternative hinge region" polypeptide sequence, which includes a polypeptide sequence that is not necessarily derived from any immunoglobulin hinge region sequence *per se*. Instead, an alternative hinge region refers to a hinge region polypeptide that comprises an amino acid

sequence, or other molecular sequence, of at least about ten consecutive amino acids or molecules, and in certain embodiments at least about 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30, 31-50, 51-60, 71-80, 81-90, or 91-110 amino acids or molecules that is present in a sequence selected from any one of SEQ ID NOS: __-__ , for example a polypeptide sequence that is or has been derived from a region located between intrachain disulfide-generated immunoglobulin-like loop domains of immunoglobulin gene superfamily members such as CD2 (*e.g.*, Genbank Acc. No. NM_001767), CD4 (*e.g.*, Genbank Acc. No. NM_000616), CD5 (*e.g.*, Genbank Acc. No. BC027901), CD6 (*e.g.*, Genbank Acc. No. NM_006725), CD7 (*e.g.*, Genbank Acc. Nos. XM_046782, BC009293, NM_006137) or CD8 (*e.g.*, Genbank Acc. No. M12828), or other Ig superfamily members. By way of non-limiting example, an alternative hinge region used as a connecting region, for example, may provide a glycosylation site as provided herein, or may provide a human gene-derived polypeptide sequence for purposes of enhancing the degree of “humanization” of a fusion protein, or may comprise, or consist essentially of, or consist of, an amino acid sequence that eliminates or reduces the ability of a construct of the invention, such as a fusion protein, to form multimers or oligomers or aggregates or the like. Certain alternative hinge region polypeptide sequences, including those described herein, may be derived or constructed from (or using) the polypeptide sequences of immunoglobulin gene superfamily members that are not actual immunoglobulins *per se*. For instance and according to non-limiting theory, certain polypeptide sequences that are situated between intrachain disulfide-generated immunoglobulin loop domain of immunoglobulin gene super-family member proteins may be used in whole or in part as alternative hinge region polypeptides as provided herein, or may be further modified for such use.

As noted above, the constructs of the invention, including binding domain-immunoglobulin fusion proteins, are believed, according to non-limiting theory, to be compromised in their ability to dimerize via interchain disulfide bond formation, and further according to theory, this property is a consequence, in whole or in part, of a reduction in the number of cysteine residues that are present in an immunoglobulin hinge region polypeptide selected for inclusion in the construction of the construct, such as a fusion protein construct. Determination of the relative ability of a polypeptide to dimerize is well within the knowledge of the relevant art, where any of a number of established

methodologies may be applied to detect protein dimerization (see, *e.g.*, Scopes, *Protein Purification: Principles and Practice*, 1987 Springer-Verlag, New York). For example, biochemical separation techniques for resolving proteins on the basis of molecular size (*e.g.*, gel electrophoresis, gel filtration chromatography, analytical ultracentrifugation, *etc.*), and/or comparison of protein physicochemical properties before and after introduction of 5 sulfhydryl-active (*e.g.*, iodoacetamide, N-ethylmaleimide) or disulfide-reducing (*e.g.*, 2-mercaptoethanol, dithiothreitol) agents, or other equivalent methodologies, may all be employed for determining a degree of polypeptide dimerization or oligomerization, and for determining possible contribution of disulfide bonds to such potential quarternary 10 structure. In certain embodiments, the invention relates to a construct, for example a binding domain-immunoglobulin fusion protein, that exhibits a reduced (*i.e.*, in a statistically significant manner relative to an appropriate IgG-derived control, for example) ability to dimerize, relative to a wild-type human immunoglobulin G hinge region polypeptide as provided herein. Those familiar with the art will be able readily to 15 determine whether a particular fusion protein displays such reduced ability to dimerize.

Compositions and methods for preparation of immunoglobulin fusion proteins, for example, are well known in the art. See, *e.g.*, U.S. Patent No. 5,892,019, which reports recombinant proteins that are the product of a single encoding polynucleotide but which are not constructs, including binding domain-immunoglobulin 20 fusion proteins, according to the present invention.

For a construct, for example, in an immunoglobulin fusion protein of the invention that is intended for use in humans, any included Ig constant regions will typically be of human sequence origin, or humanized, to minimize a potential anti-human immune response and to provide appropriate and/or desired effector functions. Manipulation of 25 sequences encoding antibody constant regions is referenced in the PCT publication of Morrison and Oi, WO 89/07142. In particularly preferred embodiments, a tail region is prepared from an immunoglobulin heavy chain constant region in which the CH1 domain is or has been deleted (the CH1 and CH2 regions in the case of IgE) and the carboxyl end of the binding domain, or where the binding domain comprises two immunoglobulin 30 variable region polypeptides, the second (*i.e.*, more proximal to the C-terminus) variable region is joined to the amino terminus of CH2 through one or more connecting regions, such as a hinge or altered region. A schematic diagram depicting the structures of two

exemplary binding domain-immunoglobulin fusion proteins is shown in FIG. 11. In particularly preferred embodiments no interchain disulfide bonds are present, and in other embodiments a restricted number of interchain disulfide bonds may be present relative to the number of such bonds that would be present if wild-type hinge region polypeptides were instead present. In other embodiments a construct of the invention, such as for example, a fusion protein, comprises, or consists essentially of, or consists of, a mutated or otherwise altered hinge region polypeptide that exhibits a reduced ability to dimerize, relative to a wild-type human IgG hinge region polypeptide. Thus, an isolated polynucleotide molecule coding for such a single chain construct, such as an immunoglobulin fusion protein, has a binding region, for example, a domain that provides specific or otherwise desired binding affinity and selectivity for a target, such as a target antigen.

The invention also contemplates, for example, in certain embodiments, constructs including binding domain-immunoglobulin fusion proteins that comprise fused or otherwise connected polypeptide sequences or portions thereof derived or prepared from a plurality of genetic sources, for example, according to molecular "domain swapping" paradigms. Those having familiarity with the art will appreciate that selection of such polypeptide sequences for assembly into a construct, such as a binding domain-immunoglobulin fusion protein, for example, may involve determination of appropriate portions of each component polypeptide sequence, for example, based on structural and/or functional properties of each such sequence (see, *e.g.*, Carayannopoulos *et al.*, 1996 *J. Exp. Med.* 183:1579; Harlow *et al.*, Eds., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988)). The component polypeptide sequences of which the construct, such as a fusion protein, is comprised or prepared may therefore comprise intact or full-length binding domain, immunoglobulin, linker and/or plasma membrane anchor domain polypeptide sequences, or truncated versions or variants thereof such as those provided herein. According to these and related embodiments of the invention, any two or more of the candidate component polypeptides of which the subject invention constructs, for example, fusion proteins, may be comprised will be derived or prepared from independent sources, such as from immunoglobulin sequences of differing allotype, isotype, subclass, class, or species of origin (*e.g.*, xenotype). Thus, as a non-limiting example, a binding domain polypeptide (or its constituent polypeptides such as

one or more variable region polypeptides and/or a linker polypeptide), a hinge region polypeptide, immunoglobulin heavy chain CH2 and CH3 constant region polypeptides and optionally an immunoglobulin heavy chain CH4 constant region polypeptide as may be obtained from an IgM or IgE heavy chain, and a plasma membrane anchor domain polypeptide may all be separately obtained from distinct genetic sources and engineered into a chimeric or fusion protein using well known techniques and according to methodologies described herein, for example.

Accordingly, a construct of the invention, for example a binding domain-immunoglobulin fusion protein according to certain embodiments of the present invention, may also therefore comprise in pertinent part an immunoglobulin heavy chain CH3 constant region polypeptide that is a wild-type IgA CH3 constant region polypeptide, or alternatively, that is a mutated or otherwise altered or substituted or truncated IgA CH3 constant region polypeptide that is incapable of associating with a J chain, or that will not associate to an undesired degree with a J chain; preferably the IgA CH3 constant region polypeptides used in a tail region portion of a construct are of human origin or are humanized. By way of brief background, IgA molecules are known to be released into secretory fluids by a mechanism that involves association of IgA into disulfide-linked polymers (*e.g.*, dimers) via a J chain polypeptide (*e.g.*, Genbank Acc. Nos. XM_059628, M12378, M12759; Johansen *et al.*, 1999 *Eur. J. Immunol.* 29:1701) and interaction of the complex so formed with another protein that acts as a receptor for polymeric immunoglobulin, and which is known as transmembrane secretory component (SC; Johansen *et al.*, 2000 *Sc. J. Immunol.* 52:240; see also, *e.g.*, Sorensen *et al.*, 2000 *Int. Immunol.* 12:19; Yoo *et al.*, 1999 *J. Biol. Chem.* 274:33771; Yoo *et al.*, 2002 *J. Immunol. Meth.* 261:1; Cortesy, 2002 *Trends Biotechnol.* 20:65; Symersky *et al.*, 2000 *Mol. Immunol.* 37:133; Crottet *et al.*, 1999 *Biochem. J.* 341:299). Interchain disulfide bond formation between IgA Fc domains and J chain is mediated through a penultimate cysteine residue in an 18-amino acid C-terminal extension that forms part of the IgA heavy chain constant region CH3 domain polypeptide (Yoo *et al.*, 1999; Sorensen *et al.*, 2000). Certain embodiments of the subject invention constructs, including for example, fusion proteins, therefore contemplate inclusion of the wild-type IgA heavy chain constant region polypeptide sequence, which is capable of associating with J chain. Certain other embodiments of the invention, however, contemplate fusion proteins that comprise a

mutated or otherwise altered, substituted, or truncated IgA CH3 constant region polypeptide that is incapable of associating with a J chain. According to such embodiments, for example, two or more residues from the C-terminus of an IgA CH3 constant region polypeptide such as a human IgA CH3 constant region polypeptide may be deleted to yield a truncated CH3 constant region polypeptide as provided herein. In preferred embodiments and as described in greater detail herein, a mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain comprises such a C-terminal deletion of either four or 18 amino acids. However, the invention need not be so limited, such that the mutated IgA CH3 constant region polypeptide may comprise a deletion of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,, 21-25, 26-30 or more amino acids, so long as the construct, for example, the fusion protein, is capable of specifically binding an antigen and of at least one immunological activity as provided herein. Alternatively, the invention also contemplates constructs, for example, fusion proteins, having a tail region that comprises a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain by virtue of replacement of the penultimate cysteine, or by chemical modification of that amino acid residue, in a manner that prevents, or inhibits an undesired level of, interchain disulfide bond or multimer formation. Methods for determining whether a construct, for example a fusion protein, can associate with a J chain will be known to those having familiarity with the art and are described or referenced herein.

As also described herein and according to procedures known in the art, the construct, for example a fusion protein, may further be tested routinely for immunological activity, for instance, in ADCC or CDC assays. As an illustrative example, a construct, for example a fusion protein, according to such an embodiment may comprise a binding domain polypeptide derived or constructed from (or using) a native or engineered human heavy chain variable region polypeptide sequence, a native or engineered human IgA-derived immunoglobulin hinge region polypeptide sequence, a native or engineered human IgG1 immunoglobulin heavy chain CH2 constant region polypeptide sequence, a native or engineered human IgG2 immunoglobulin heavy chain CH3 constant region polypeptide sequence, and optionally a native or engineered human IgE immunoglobulin heavy chain CH4 constant region polypeptide sequence and/or a native or engineered human TNF- α receptor type 1 (TNFR1) plasma membrane anchor domain polypeptide sequence that

comprises a cytoplasmic tail polypeptide which is capable of apoptotic signaling or otherwise promoting apoptosis. The invention therefore contemplates these and other embodiments according to the present invention in which two or more polypeptide sequences that are present in a construct, for example a fusion protein, have independent
5 genetic origins.

As noted above, in certain embodiments the construct, for example a binding protein-immunoglobulin fusion protein, comprises at least one native or engineered immunoglobulin variable region polypeptide, which may be a native or engineered light chain or a native or engineered heavy chain variable region polypeptide,
10 and in certain embodiments the fusion protein comprises at least one such native or engineered light chain V-region and one such native or engineered heavy chain V-region and at least one linker peptide that is fused or otherwise connected to each of the native or engineered V-regions. Construction of such binding domains, for example single chain Fv domains, is known in the art and is described in greater detail in the Examples below,
15 and has been described, for example, in various documents cited herein; selection and assembly of single-chain variable regions and of linker polypeptides that may be fused or otherwise connected to each of a heavy chain-derived and a light chain-derived V region (e.g., to generate a binding region, such as a binding domain that comprises a single-chain Fv polypeptide) is also known to the art and described herein. See, e.g., U.S. Patent Nos.
20 5,869,620, 4,704,692, and 4,946,778. In certain embodiments all or a portion or portions of an immunoglobulin sequence that is derived from a non-human source may be "humanized" according to recognized procedures for generating humanized antibodies, i.e., immunoglobulin sequences into which human Ig sequences are introduced to reduce the degree to which a human immune system would perceive such proteins as foreign (see,
25 e.g., U.S. Patent Nos. 5,693,762; 5,585,089; 4,816,567; 5,225,539; 5,530,101; and documents cited therein).

Constructs of the invention, including binding domain-immunoglobulin fusion proteins, as described herein may, according to certain embodiments, desirably comprise sites for glycosylation, e.g., covalent attachment of carbohydrate moieties such
30 as, for example, monosaccharides or oligosaccharides. Incorporation of amino acid sequences that provide substrates for polypeptide glycosylation is within the scope of the relevant art, including, for example, the use of genetic engineering or protein engineering

methodologies to obtain a polypeptide sequence containing, for example, the classic Asn-X-Ser/Thr site for N-(asparagine)-linked glycosylation, or a sequence containing Ser or Thr residues that are suitable substrates for O-linked glycosylation, or sequences amenable to C-mannosylation, glypiation/glycosylphosphatidylinositol modification, or phosphoglycation, all of which can be identified according to art-established criteria (e.g., Spiro, 2002 *Glyobiology* 12:43R). Without wishing to be bound by any particular theory or mechanism, glycosylated constructs such as fusion proteins having particular amino acid sequences may beneficially possess attributes associated with one or more of improved solubility, enhanced stability in solution, enhanced physiological stability, improved bioavailability including in vivo biodistribution, and superior resistance to proteases, all in a statistically significant manner, relative to constructs, including fusion proteins, having the same or highly similar amino acid sequences but lacking glycosyl moieties. In certain preferred embodiments the subject invention constructs, such as fusion protein constructs, may comprise a glycosylation site that is present in a linker as provided herein, and in certain other preferred embodiments the subject invention construct, for example, a fusion protein, comprises a glycosylation site that is present in a connecting region, such as a hinge region polypeptide sequence as provided herein.

In certain preferred embodiments of the present invention, such as those useful for gene therapy applications or in display systems or assays, such as screening assays (including library display systems and library screening assays), the construct, for example, a binding domain-immunoglobulin fusion protein, is a protein or glycoprotein that is capable of being expressed by a host cell such that it localizes to the cell surface. Constructs, such as binding domain-immunoglobulin fusion proteins, that localize to the cell surface may do so by virtue of having naturally present or artificially introduced structural features that direct the fusion protein to the cell surface (e.g., Nelson *et al.* 2001 *Trends Cell Biol.* 11:483; Ammon *et al.*, 2002 *Arch. Physiol. Biochem.* 110:137; Kasai *et al.*, 2001 *J. Cell Sci.* 114:3115; Watson *et al.*, 2001 *Am. J. Physiol. Cell Physiol.* 281:C215; Chatterjee *et al.*, 200 *J. Biol. Chem.* 275:24013) including by way of illustration and not limitation, secretory signal sequences, leader sequences, plasma membrane anchor domain polypeptides and transmembrane domains such as hydrophobic transmembrane domains (e.g., Heuck *et al.*, 2002 *Cell Biochem. Biophys.* 36:89; Sadlish *et al.*, 2002 *Biochem J.* 364:777; Phoenix *et al.*, 2002 *Mol. Membr. Biol.* 19:1; Minke *et al.*, 2002 *Physiol. Rev.*

82:429) or glycosylphosphatidylinositol attachment sites ("glypiation" sites, *e.g.*, Chatterjee *et al.*, 2001 *Cell Mol. Life Sci.* 58:1969; Hooper, 2001 *Proteomics* 1:748; Spiro, 2002 *Glycobiol.* 12:43R), cell surface receptor binding domains, extracellular matrix binding domains, or any other structural feature that causes at least a desired portion of the fusion protein population to localize, in whole or in part, to the cell surface. Particularly preferred are fusion protein constructs that comprise a plasma membrane anchor domain which includes a transmembrane polypeptide domain, typically comprising a membrane spanning domain which includes a hydrophobic region capable of energetically favorable interaction with the phospholipid fatty acyl tails that form the interior of the plasma membrane bilayer. Such features are known to those of ordinary skill in the art, who will further be familiar with methods for introducing nucleic acid sequences encoding these features into the subject expression constructs by genetic engineering, and with routine testing of such constructs to verify cell surface localization of the product.

According to certain further embodiments, a plasma membrane anchor domain polypeptide comprises such a transmembrane domain polypeptide and also comprises a cytoplasmic tail polypeptide, which refers to a region or portion of the polypeptide sequence that contacts the cytoplasmic face of the plasma membrane and/or is in contact with the cytosol or other cytoplasmic components. A large number of cytoplasmic tail polypeptides are known that comprise the intracellular portions of plasma membrane transmembrane proteins, and discrete functions have been identified for many such polypeptides, including biological signal transduction (*e.g.*, activation or inhibition of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides and other second messengers, ion channels, secretory pathways), biologically active mediator release, stable or dynamic association with one or more cytoskeletal components, cellular differentiation, cellular activation, mitogenesis, cytostasis, apoptosis and the like (*e.g.*, Maher *et al.*, 2002 *Immunol. Cell Biol.* 80:131; El Far *et al.*, 2002 *Biochem J.* 365:329; Teng *et al.*, 2002 *Genome Biol.* 2REVIEWS:3012; Simons *et al.*, 2001 *Cell Signal* 13:855; Furie *et al.*, 2001 *Thromb. Haemost.* 86:214; Gaffen, 2001 *Cytokine* 14:63; Dittel, 2000 *Arch. Immunol. Ther. Exp. (Warsz.)* 48:381; Parnes *et al.*, 2000 *Immunol. Rev.* 176:75; Moretta *et al.*, 2000 *Semin. Immunol.* 12:129; Ben Ze'ev, 1999 *Ann. N.Y. Acad. Sci.* 886:37; Marsters *et al.*, 2000 *Recent Prog. Horm. Res.* 54:225).

Figure 70 illustrates the binding, for example, of fluorceine-conjugated FcR^{III} (CD16) soluble fusion proteins to 2H7 scFv-binding domain constructs that are attached to CD20 expressed by cells, CHO cells in this example. CD16 binding to a construct of the invention, for example, scFv-binding domain construct, provides one
5 example of a screening tool that may be used to detect and/or quantitate changes in CD16 binding to altered constructs of the invention, including scFv-binding domain constructs, that contains targeted or site-specific mutations, substitutions, deletions, or other alterations. Changes in CD16 binding properties may be reflected, for example, by changes in binding of either CD16 high affinity protein (158V) or CD16 low affinity
10 protein (158 F) or both.

A schematic representation of one example of such a screening process is diagrammed in the second drawing in Figure 70, in which scFv-binding domain constructs are displayed on the cell surface of mammalian cells. scFv-binding domain molecules in this example are displayed on the cell surface through a molecule that serves as a
15 transmembrane domain anchor. These molecules may represent, for example, a single scFv-binding domain construct or may be introduced into a population of mammalian cells as a library of such molecules. Transfected cells with altered binding properties can then, for example, be panned, sorted, or otherwise isolated from other cells by altering the stringency of the selection conditions and using CD16 fusion proteins as a binding probe.
20 Cells that express scFv-Ig molecules with altered binding to either CD16 high affinity allele (158V) or CD16 low affinity allele (158F) or both, for example, can be isolated.

This display system can be used, for example, to create a library of constructs of the invention with mutated or otherwise altered tail regions with short stretches of CH2 sequence replaced with randomized oligonucleotides or, for example,
25 randomization of a single residue with all possible amino acid substitutions, natural or unnatural, including synthetic amino acids. Once such a library is constructed, it can be transfected into appropriate cells, for example, COS cells, by methods known in the art. Transfectants can then be bound to, for example, labeled CD16 constructs, and panned or sorted based on their relative or desired binding properties to multiple allelotypes/isoforms.
30 Desired cells may be harvested, and the DNA, for example, plasmid DNA, isolated and then transformed into, for example, bacteria. This process may be repeated iteratively multiple times until desired single clones are isolated from the mammalian host cells. See

Seed B and Aruffo A, *Pro. Nat'l Acad Sci USA* 1987 84:3365-3369; Aruffo A and Seed B, *Pro. Nat'l Acad Sci USA* 1987 84:8573-8577.

One such use of this type of screening system, for example, is for the identification and/or isolation of constructs of the invention having tail regions, or tail regions, that bind equally well to both the high and low affinity alleles of CD16 with the goal of improving effector functions mediated by scFv-binding domain constructs in multiple subpopulations of patients. Constructs of the invention having tail regions, or tail regions with altered binding properties to other Fc receptors can also be selected using such a display system, for example, the display system described. Other display systems that do not glycosylate proteins, for example, those that use bacteriophage or yeast, are not generally desired for selection of constructs of the invention having Ig-based tail regions, or Ig-based tail regions, with altered FcR binding properties. Most non-mammalian systems, for example, do not glycosylate proteins.

Expression of constructs of the invention, for example, scFv-binding domain constructs, expressed on the surface of a mammalian cell by incorporation of an appropriate molecule into the construct, for example, by incorporation of a transmembrane domain or a GPI anchor signal, also have utility in other display systems that are useful, for example, for selection of constructs of the invention, for example, altered scFv-binding domain molecules that will be produced at higher or other desired levels. In one such an embodiment, cells that are useful in the production of glycosylated proteins, for example, mammalian cells such as COS cells, are transfected with a library of scFv-binding domain constructs in a plasmid that directs their expression to the cell surface. Cells, such as COS cells, that express the highest or other desired level of the scFv-binding domain molecules are selected by techniques known in the art (for example panning, sterile cell sorting, magnetic bead separation, *etc.*), and DNA, for example, plasmid DNA, is isolated for transformation into other cells, for example, bacteria. After one or more rounds of selection single clones are isolated that encode scFv-binding domain molecules capable of a high or other desired level of expression. The isolated clones may then be altered to remove the membrane anchor and expressed in an appropriate cells system, for example, a mammalian cell system, wherein the scFv-binding domain constructs will be produced, for example, by secretion into the culture fluid at desired levels. Without being bound by any particular mechanism or theory, this is believed to result from the common requirement of

secreted glycoproteins and cell surface glycoproteins for a signal peptide and processing through the golgi for expression. Thus, selection for a molecule that shows an improvement expression levels on a cell surface will also result in the identification of a molecule having an improvement in levels of secreted protein.

5 These display systems utilizing a construct of the invention may also be used for screening and/or identifying and/or isolating affinity variants of the binding domain within a construct.

 Particularly preferred are such display and/or screening systems, for example, that include or use constructs that include (1) an immunoglobulin variable region
10 polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, in a V_H region sequence (including in a V_H region sequence within an scFv or other construct), and/or (2) an immunoglobulin
15 variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence (including in a V_L region sequence within an scFv or other construct). Especially
20 preferred are such display and/or screening systems that include or use constructs that include an engineered V_H sequence (whether or not associated with one or more other sequences, including immunoglobulin-derived and other sequences contained, for example, within an sFv or scFv-containing construct), which includes a mutation, alteration or deletion at an amino acid at a location or locations corresponding to amino acid position
25 11. The V_H11 amino acid, if substituted, may be substituted with another amino acid as described herein, or by another molecule as desired.

 In the context of other methods of using constructs of the invention, including binding domain-immunoglobulin fusion proteins, for the treatment of a malignant condition or a B cell disorder(s) as provided herein, including, for example, by
30 one or more of a number of gene therapy methods and related construct delivery techniques, the present invention also contemplates certain embodiments wherein a construct, for example, a binding domain-immunoglobulin fusion protein that comprises a

plasma membrane anchor domain polypeptide is expressed (or capable of expression) at a cell surface and may further comprise a cytoplasmic tail polypeptide which comprises an apoptosis signaling polypeptide sequence. A number of apoptosis signaling polypeptide sequences are known to the art, as reviewed, for example, in *When Cells Die: A Comprehensive Evaluation of Apoptosis and Programmed Cell Death* (R.A. Lockshin *et al.*, Eds., 1998 John Wiley & Sons, New York; see also, *e.g.*, Green *et al.*, 1998 *Science* 281:1309 and references cited therein; Ferreira *et al.*, 2002 *Clin. Canc. Res.* 8:2024; Gurumurthy *et al.*, 2001 *Cancer Metastas. Rev.* 20:225; Kanduc *et al.*, 2002 *Int. J. Oncol.* 21:165). Typically an apoptosis signaling polypeptide sequence comprises all or a portion of, or is derived or constructed from, a receptor death domain polypeptide, for instance, FADD (*e.g.*, Genbank Acc. Nos. U24231, U43184, AF009616, AF009617, NM_012115), TRADD (*e.g.*, Genbank Acc. No. NM_003789), RAIDD (*e.g.*, Genbank Acc. No. U87229), CD95 (FAS/Apo-1; *e.g.*, Genbank Acc. Nos. X89101, NM_003824, AF344850, AF344856), TNF- α -receptor-1 (TNFR1, *e.g.*, Genbank Acc. Nos. S63368, AF040257), DR5 (*e.g.*, Genbank Acc. No. AF020501, AF016268, AF012535), an ITIM domain (*e.g.*, Genbank Acc. Nos. AF081675, BC015731, NM_006840, NM_006844, NM_006847, XM_017977; see, *e.g.*, Billadeau *et al.*, 2002 *J. Clin. Invest.* 109:161), an ITAM domain (*e.g.*, Genbank Acc. Nos. NM_005843, NM_003473, BC030586; see, *e.g.*, Billadeau *et al.*, 2002), or other apoptosis-associated receptor death domain polypeptides known to the art, for example, TNFR2 (*e.g.*, Genbank Acc. No. L49431, L49432), caspase/procaspase-3 (*e.g.*, Genbank Acc. No. XM_54686), caspase/procaspase-8 (*e.g.*, AF380342, NM_004208, NM_001228, NM_033355, NM_033356, NM_033357, NM_033358), caspase/procaspase-2 (*e.g.*, Genbank Acc. No. AF314174, AF314175), *etc.*

Cells in biological samples that are suspected of undergoing apoptosis may be examined for morphological, permeability or other changes that are indicative of an apoptotic state. For example by way of illustration and not limitation, apoptosis in many cell types may cause altered morphological appearance such as plasma membrane blebbing, cell shape change, loss of substrate adhesion properties or other morphological changes that can be readily detected by a person having ordinary skill in the art, for example by using light microscopy. As another example, cells undergoing apoptosis may exhibit fragmentation and disintegration of chromosomes, which may be apparent by microscopy and/or through the use of DNA-specific or chromatin-specific dyes that are

known in the art, including fluorescent dyes. Such cells may also exhibit altered plasma membrane permeability properties as may be readily detected through the use of vital dyes (e.g., propidium iodide, trypan blue) or by the detection of lactate dehydrogenase leakage into the extracellular milieu. These and other means for detecting apoptotic cells by morphologic criteria, altered plasma membrane permeability, and related changes will be apparent to those familiar with the art.

In another embodiment of the invention wherein a construct, such as a binding domain-immunoglobulin fusion protein, that is expressed at a cell surface comprises a plasma membrane anchor domain having a transmembrane domain and a cytoplasmic tail that comprises an apoptosis signaling polypeptide, cells in a biological sample may be assayed for translocation of cell membrane phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane, which may be detected, for example, by measuring outer leaflet binding by the PS-specific protein annexin. Martin *et al.*, *J. Exp. Med.* **182**:1545, 1995; Fadok *et al.*, *J. Immunol.* **148**:2207, 1992. In still other related embodiments of the invention, including embodiments wherein a construct, such as a binding domain-immunoglobulin fusion protein, is expressed at the cell surface and comprises a plasma membrane anchor domain having an apoptosis signaling polypeptide and also including embodiments wherein the construct, such as a binding domain-immunoglobulin fusion protein, is a soluble protein that lacks a membrane anchor domain and that is capable of inducing apoptosis, a cellular response to an apoptogen is determined by an assay for induction of specific protease activity in any member of a family of apoptosis-activated proteases known as the caspases (see, e.g., Green *et al.*, 1998 *Science* **281**:1309). Those having ordinary skill in the art will be readily familiar with methods for determining caspase activity, for example by determination of caspase-mediated cleavage of specifically recognized protein substrates. These substrates may include, for example, poly-(ADP-ribose) polymerase (PARP) or other naturally occurring or synthetic peptides and proteins cleaved by caspases that are known in the art (see, e.g., Ellerby *et al.*, 1997 *J. Neurosci.* **17**:6165). The synthetic peptide Z-Tyr-Val-Ala-Asp-AFC (SEQ ID NO:___), wherein "Z" indicates a benzoyl carbonyl moiety and AFC indicates 7-amino-4-trifluoromethylcoumarin (Kluck *et al.*, 1997 *Science* **275**:1132; Nicholson *et al.*, 1995 *Nature* **376**:37), is one such substrate. Other non-limiting examples of substrates include nuclear proteins such as U1-70 kDa and DNA-PKcs (Rosen and Casciola-Rosen, 1997 *J.*

Cell. Biochem. 64:50; Cohen, 1997 *Biochem. J.* 326:1). Cellular apoptosis may also be detected by determination of cytochrome c that has escaped from mitochondria in apoptotic cells (e.g., Liu *et al.*, *Cell* 86:147, 1996). Such detection of cytochrome c may be performed spectrophotometrically, immunochemically or by other well established methods for determining the presence of a specific protein. Persons having ordinary skill in the art will readily appreciate that there may be other suitable techniques for quantifying apoptosis.

Particularly preferred embodiments of constructs useful for gene therapy applications, including those constructs that include a plasma membrane anchor domain and/or cytoplasmic tail polypeptide (including, for example, an apoptosis signaling sequence), are such constructs that include (1) an immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, in a V_H region sequence (including in a V_H region sequence within an scFv or other construct), and/or (2) an immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence (including in a V_L region sequence within an scFv or other construct). Especially preferred are constructs that include an engineered V_H sequence (whether or not associated with one or more other sequences, including immunoglobulin-derived and other sequences contained, for example, within an sFv or scFv-containing construct), which includes a mutation, alteration or deletion at an amino acid at a location or locations corresponding to amino acid position 11. The V_H11 amino acid, if substituted, may be substituted with another amino acid as described herein, or by another molecule as desired.

Once a construct, such as for example a binding domain-immunoglobulin fusion protein, as provided herein has been designed, polynucleotides including DNAs encoding the construct, where it or a relevant portion of it is a polypeptide, may be synthesized in whole or in part via oligonucleotide synthesis as described, for example, in Sinha *et al.*, *Nucleic Acids Res.*, 12:4539-4557 (1984); assembled via PCR as described, for

example in Innis, Ed., *PCR Protocols*, Academic Press (1990) and also in Better *et al.* *J. Biol. Chem.* 267:16712-16118 (1992); cloned and expressed via standard procedures as described, for example, in Ausubel *et al.*, Eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1989) and also in Robinson *et al.*, *Hum. Antibod. Hybridomas*, 2:84-93 (1991); and tested for desired activity, for example, binding to a target, or specific antigen binding activity, as described, for example, in Harlow *et al.*, Eds., *Antibodies: A Laboratory Manual*, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988) and Munson *et al.*, *Anal. Biochem.*, 107:220-239 (1980).

The preparation of single polypeptide chain binding molecules of the Fv region, single-chain Fv molecules, is known in the art. See, *e.g.*, U.S. Pat. No. 4,946,778. In the present invention, single-chain Fv-like molecules that may be included in constructs of the invention may be synthesized by encoding a first variable region of the heavy or light chain, followed by one or more linkers to the variable region of the corresponding light or heavy chain, respectively. The selection of various appropriate linker(s) between the two variable regions is described in U.S. Pat. No. 4,946,778 (see also, *e.g.*, Huston *et al.*, 1993 *Int. Rev. Immunol.* 10:195). An exemplary linker described herein is (Gly-Gly-Gly-Gly-Ser)₃, but may be of any desired length. The linker is used to convert the naturally aggregated but chemically separate heavy and light chains into the amino terminal antigen binding portion of a single polypeptide chain, for example, wherein this antigen binding portion will fold into a structure similar to the original structure made of two polypeptide chains, or that otherwise has the ability to bind to a target, for example a target antigen. For those constructs that include an scFv as a binding region, a native or engineered immunoglobulin hinge as a connecting region, and one or more native or engineered heavy chain constant regions as a binding region, nucleotide sequences encoding the variable regions of native or engineered heavy and light chains, joined by a sequence encoding a linker, are joined to a nucleotide sequence encoding native or engineered antibody constant regions, as desired. The constant regions may be those that permit the resulting polypeptide to form interchain disulfide bonds to form a dimer, and which contain desired effector functions, such as the ability to mediate ADCC, CDC, or fix complement, although native or engineered constant regions that do not favor dimer or other multimer formation or aggregation are preferred. For a construct, such as an immunoglobulin-like molecule, of the invention that is intended for use in humans, the

included sequences having constant regions and/or desired constant regions function(s) will typically be human or substantially human or humanized to minimize a potential anti-human immune response and to provide appropriate or desired effector functions. Manipulation of sequences encoding antibody constant regions is referenced in the PCT publication of Morrison and Oi, WO 89/07142. In preferred embodiments, the CH1 domain is deleted in whole or in part from a tail region that includes, or consists essentially of, or consists of, a native or engineered immunoglobulin constant region(s) (for example, native or engineered CH2 and/or CH3 constant region(s), or native or engineered CH2 and/or CH3 and/or CH4 constant region(s)), and the carboxyl end of the binding region, for example, a binding domain polypeptide such as an immunoglobulin variable region polypeptide, is joined to the amino terminus of, for example, a CH2 via a connecting region, for example, a native or engineered hinge region polypeptide as provided herein.

As described above, the present invention provides recombinant expression constructs capable of directing the expression of constructs of the invention, including binding domain-immunoglobulin fusion proteins, as provided herein. The amino acids, which occur in the various amino acid sequences referred to herein, are identified according to their well known three-letter or single-letter abbreviations. The nucleotides, which occur in the various DNA sequences or fragments thereof referred herein, are designated with the standard single letter designations used routinely in the art. A given amino acid sequence may also encompass similar but changed amino acid sequences, such as those having only minor changes, for example by way of illustration and not limitation, covalent chemical modifications, insertions, deletions and substitutions, which may further include conservative substitutions or substitutions with non-naturally-occurring amino acids. Amino acid sequences that are similar to one another may share substantial regions of sequence homology. In like fashion, nucleotide sequences may encompass substantially similar nucleotide sequences having only minor changes, for example by way of illustration and not limitation, covalent chemical modifications, insertions, deletions and substitutions, which may further include silent mutations owing to degeneracy of the genetic code. Nucleotide sequences that are similar to one another may share substantial regions of sequence homology.

The presence of a malignant condition in a subject refers to the presence of dysplastic, cancerous, and/or transformed cells in the subject, including, for example,

neoplastic, tumor, non-contact inhibited, or oncogenically transformed cells, or the like (e.g., melanoma, carcinomas such as adenocarcinoma, squamous cell carcinoma, small cell carcinoma, oat cell carcinoma, *etc.*, sarcomas such as chondrosarcoma, osteosarcoma, *etc.*) which are known to the art and for which criteria for diagnosis and classification are established. In preferred embodiments contemplated by the present invention, for example, such cancer cells are malignant hematopoietic cells, such as transformed cells of lymphoid lineage and in particular, B cell lymphomas and the like; cancer cells may in certain preferred embodiments also be epithelial cells such as carcinoma cells. The invention also contemplates B cell disorders, which may include certain malignant conditions that affect B cells (e.g., B cell lymphoma) but which is not intended to be so limited, and which is also intended to encompass autoimmune diseases and in particular, diseases, disorders and conditions that are characterized by autoantibody production, for example.

Autoantibodies are antibodies that react with self-antigens. Autoantibodies are detected in several autoimmune diseases (*i.e.*, a disease, disorder or condition wherein a host immune system generates an inappropriate anti-“self” immune reaction) where they are involved in disease activity. Current treatments for various autoimmune diseases include immunosuppressive drugs that require continuing administration, lack specificity, and cause significant side effects. New approaches that can eliminate autoantibody production with minimal toxicity will address an unmet medical need for a spectrum of diseases that affect many people. Constructs of the subject invention, including binding domain-immunoglobulin fusion proteins, are designed, for example, for improved penetration into lymphoid tissues. Depletion of B lymphocytes interrupts the autoantibody production cycle, and allows the immune system to reset as new B lymphocytes are produced from precursors in the bone marrow.

A number of diseases, disorders, and conditions have been identified for which beneficial effects are believed, according to non-limiting theory, to result from B cell depletion therapy. Such diseases disorders, and conditions include, but are not limited to, Grave's disease, Hashimoto's disease, rheumatoid arthritis, systemic lupus erythematosus, Sjogrens Syndrome Immune Thrombocytopenic purpura, multiple sclerosis, myasthenia gravis, scleroderma, psoriasis, Inflammatory Bowel Disease including Crohn's disease and ulcerative colitis. Inflammatory Bowel Disease including Crohn's disease and Ulcerative colitis, are autoimmune diseases of the digestive system.

The present invention further relates to nucleotide constructs encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, and in particular to methods for administering recombinant constructs encoding such proteins for gene therapy applications that may be expressed, for example, as fragments,
5 analogs and derivatives of such polypeptides.

The terms "fragment," "derivative" and "analog" when referring to constructs of the invention including, for example, binding domain-immunoglobulin fusion polypeptides or fusion proteins, refers to any construct, such as a binding domain-immunoglobulin fusion polypeptide or fusion protein, that retains essentially the same
10 biological function or activity as such polypeptide. Thus, an analog includes a pro- or prepro- form of a construct, for example, a pro-protein that can be activated by cleavage of the pro-protein portion to produce an active construct, such as a binding domain-immunoglobulin fusion polypeptide.

A fragment, derivative or analog of a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide or fusion protein,
15 including binding domain-immunoglobulin fusion polypeptides or fusion proteins encoded by the cDNAs referred to herein, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not
20 be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which additional amino acids are fused or otherwise connected to the construct, *e.g.*, a binding domain-immunoglobulin fusion polypeptide, including amino acids that are employed for detection or specific functional alteration of the construct, including such constructs as a binding domain-immunoglobulin
25 fusion polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The constructs, including polypeptide constructs, of the present invention include, for example, binding domain-immunoglobulin fusion polypeptides and fusion proteins having binding regions such as binding domain polypeptide amino acid sequences
30 that are identical or similar to sequences known in the art, or fragments or portions thereof. For example by way of additional illustration and not limitation, a human CD154 molecule extracellular domain [SEQ ID NO:___] is contemplated for use according to the instant

invention, as are portions of such polypeptides and/or polypeptides having at least about 70% similarity (preferably greater than a 70% identity) and more preferably about 90% similarity (more preferably greater than a 90% identity) to the reported polypeptide and still more preferably about 95% similarity (still more preferably greater than a 95% identity) to the reported polypeptides and to portions of such polypeptides, wherein such portions of a binding domain-immunoglobulin fusion polypeptide, for example, generally contain at least about 30 amino acids and more preferably at least about 50 amino acids. Extracellular domains include, for example, portions of a cell surface molecule, and in particularly preferred embodiments cell surface molecules that are integral membrane proteins or that comprise a plasma membrane spanning transmembrane domain, that are constructed to extend beyond the outer leaflet of the plasma membrane phospholipid bilayer when the molecule is expressed at a cell surface, preferably in a manner that exposes the extracellular domain portion of such a molecule to the external environment of the cell, also known as the extracellular milieu. Methods for determining whether a portion of a cell surface molecule comprises an extracellular domain are well known to the art and include, for example, experimental determination (*e.g.*, direct or indirect labeling of the molecule, evaluation of whether the molecule can be structurally altered by agents to which the plasma membrane is not permeable such as proteolytic or lipolytic enzymes) or topological prediction based on the structure of the molecule (*e.g.*, analysis of the amino acid sequence of a polypeptide) or other methodologies.

As used herein, an "amino acid" is a molecule having the structure wherein a central carbon atom (the alpha (α)-carbon atom) is linked to a hydrogen atom, a carboxylic acid group (the carbon atom of which is referred to herein as a "carboxyl carbon atom"), an amino group (the nitrogen atom of which is referred to herein as an "amino nitrogen atom"), and a side chain group, R. When incorporated into a peptide, polypeptide, or protein, an amino acid loses one or more atoms of its amino and carboxylic groups in the dehydration reaction that links one amino acid to another. As a result, when incorporated into a protein, an amino acid may also be referred to as an "amino acid residue." In the case of naturally occurring proteins, an amino acid residue's R group differentiates the amino acids from which proteins are typically synthesized, although one or more amino acid residues in a protein may be derivatized or modified following incorporation into protein in biological systems (*e.g.*, by glycosylation and/or by the formation of cystine

through the oxidation of the thiol side chains of two non-adjacent cysteine amino acid residues, resulting in a disulfide covalent bond that frequently plays an important role in stabilizing the folded conformation of a protein, *etc.*). As those in the art will appreciate, non-naturally occurring amino acids can also be incorporated into proteins, particularly those produced by synthetic methods, including solid state and other automated synthesis methods. Examples of such amino acids include, without limitation, α -amino isobutyric acid, 4-amino butyric acid, L-amino butyric acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norlensine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids (*e.g.*, β -methyl amino acids, α -methyl amino acids, N α -methyl amino acids) and amino acid analogs in general. In addition, when an α -carbon atom has four different groups (as is the case with the 20 amino acids used by biological systems to synthesize proteins, except for glycine, which has two hydrogen atoms bonded to the α carbon atom), two different enantiomeric forms of each amino acid exist, designated D and L. In mammals, only L-amino acids are incorporated into naturally occurring polypeptides. The instant invention envisions proteins incorporating one or more D- and L- amino acids, as well as proteins comprised of just D- or L- amino acid residues.

Herein, the following abbreviations may be used for the following amino acids (and residues thereof): alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); cyteine (Cys, C); glycine (Gly, G); glutamic acid (Glu, E); glutamine (Gln, Q); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y); and valine (Val, V). Non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionines. Neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, esparagine, and glutamine. Positively charged (basic amino acids include arginine, lysine and histidine. Negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

“Protein” refers to any polymer of two or more individual amino acids (whether or not naturally occurring) linked via a peptide bond, and occurs when the carboxyl carbon atom of the carboxylic acid group bonded to the α -carbon of one amino

acid (or amino acid residue) becomes covalently bound to the amino nitrogen atom of amino group bonded to the α -carbon of an adjacent amino acid. The term "protein" is understood to include the terms "polypeptide" and "peptide" (which, at times, may be used interchangeably herein) within its meaning. In addition, proteins comprising multiple polypeptide subunits or other components will also be understood to be included within the meaning of "protein" as used herein. Similarly, fragments of proteins, peptides, and polypeptides are also within the scope of the invention and may be referred to herein as "proteins."

In biological systems (be they *in vivo* or *in vitro*, including cell-free, systems), the particular amino acid sequence of a given protein (*i.e.*, the polypeptide's "primary structure," when written from the amino-terminus to carboxy-terminus) is determined by the nucleotide sequence of the coding portion of a mRNA, which is in turn specified by genetic information, typically genomic DNA (which, for purposes of this invention, is understood to include organelle DNA, for example, mitochondrial DNA and chloroplast DNA). Of course, any type of nucleic acid which constitutes the genome of a particular organism (*e.g.*, double-stranded DNA in the case of most animals and plants, single or double-stranded RNA in the case of some viruses, *etc.*) is understood to code for the gene product(s) of the particular organism. Messenger RNA is translated on a ribosome, which catalyzes the polymerization of a free amino acid, the particular identity of which is specified by the particular codon (with respect to mRNA, three adjacent A, G, C, or U ribonucleotides in the mRNA's coding region) of the mRNA then being translated, to a nascent polypeptide. Recombinant DNA techniques have enabled the large-scale synthesis of proteins and polypeptides (*e.g.*, human insulin, human growth hormone, erythropoietin, granulocyte colony stimulating factor, *etc.*) having the same primary sequence as when produced naturally in living organisms. In addition, such technology has allowed the synthesis of analogs of these and other proteins, which analogs may contain one or more amino acid deletions, insertions, and/or substitutions as compared to the native proteins. Recombinant DNA technology also enables the synthesis of entirely novel proteins.

In non-biological systems (*e.g.*, those employing solid state synthesis), the primary structure of a protein (which also includes disulfide (cystine) bond locations) can be determined by the user. As a result, polypeptides having a primary structure that

duplicates that of a biologically produced protein can be achieved, as can analogs of such proteins. In addition, completely novel polypeptides can also be synthesized, as can protein incorporating non-naturally occurring amino acids.

As is known in the art, "similarity" between two polypeptides may be
5 determined by comparing the amino acid sequence (including conserved amino acid
substitutes therein) of one polypeptide to the sequence of a second polypeptide. Fragments
or portions of the nucleic acids encoding polypeptides of the present invention may be used
to synthesize full-length nucleic acids of the present invention. As used herein, "%
identity" refers to the percentage of identical amino acids situated at corresponding amino
10 acid residue positions when two or more polypeptide are aligned and their sequences
analyzed using, for example, a gapped BLAST algorithm (*e.g.*, Altschul *et al.*, 1997 *Nucl.*
Ac. Res. **25**:3389; Altschul *et al.*, 1990 *J. Mol. Biol.*, **215**:403-410) which weights sequence
gaps and sequence mismatches according to the default weightings provided by the
National Institutes of Health/ NCBI database (Bethesda, MD; *see*
15 www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast). Other alignment methods include
BLITZ (MPsrch) (Sturrock & Collins, 1993), and FASTA (Pearson and Lipman, 1988
Proc. Natl. Acad. Sci. USA. **85**:2444-2448).

The term "isolated" means, in the case of a naturally occurring material, that
the material is or has been removed from, or is no longer associated with, its natural or
20 original environment. For example, a naturally occurring nucleic acid or protein or
polypeptide present in a living animal is not isolated, but the same nucleic acid or
polypeptide, separated from some or all of the co-existing materials in the natural system,
is isolated. Such nucleic acids could be part of a vector and/or such nucleic acids or
polypeptides could be part of a composition, and still be isolated in that such vector or
25 composition is not part of its natural environment. The term "isolated", in the case of non-
naturally occurring material, such as a recombinantly manufactured construct of the
invention, includes material that is substantially or essentially free from components which
normally accompany it during manufacture, such as, for example, proteins and peptides
that have been purified to a desired degree, preferably, for example, so that they are at least
30 about 80% pure, more preferably at least about 90%, and still more preferably at least
about 95% as measured by techniques known in the art.

The term “gene” means a segment of DNA involved in producing a polypeptide chain; it may also include regions preceding and following a polypeptide coding region, for example, a “leader and trailer” as well as intervening sequences (introns) between relevant individual coding segments (exons).

5 As described herein, the invention provides constructs, including binding domain-immunoglobulin fusion proteins, that may be encoded in whole or in part by nucleic acids that have a binding region coding sequence such as, for example, a binding domain coding sequence fused or otherwise connected in frame to an additional native or engineered immunoglobulin domain encoding sequence to provide for expression of, for
10 example, a binding domain polypeptide sequence fused or otherwise connected to an additional functional polypeptide sequence that permits, for example by way of illustration and not limitation, detection, functional alteration, isolation and/or purification of the fusion protein. Such fusion proteins may permit functional alteration of a binding domain by containing additional immunoglobulin-derived polypeptide sequences that influence
15 behavior of the fusion product, for example (and as described above) by reducing the availability of sufhydryl groups for participation in disulfide bond formation, and by conferring the ability to potentiate ADCC and/or CDC and/or fix complement.

 Modification of a polypeptide may be effected by any means known to those of skill in this art. The preferred methods herein rely on modification of DNA
20 encoding, for example, a fusion protein and expression of the modified DNA. DNA encoding one of the constructs of the invention, for example, one of the binding domain-immunoglobulin fusions discussed herein, for example, may be altered or mutagenized using standard methodologies, including those described below. For example, cysteine residues that may otherwise facilitate multimer formation or promote particular molecular
25 conformations can be deleted from a polypeptide or replaced, *e.g.*, cysteine residues that are responsible for or participate in aggregate formation. If necessary, for example, the identity of cysteine residues that contribute to aggregate formation may be determined empirically, by deleting and/or replacing a cysteine residue and ascertaining whether the resulting protein aggregates in solutions containing physiologically acceptable buffers and
30 salts. In addition, fragments of, for example, binding domain-immunoglobulin fusions may be constructed and used. As noted above, counterreceptor/ligand binding domains for many candidate binding domain-immunoglobulin fusion have been delineated, such that

one having ordinary skill in the art may readily select appropriate polypeptide domains for inclusion in encoded products of the instant expression constructs.

Conservative substitutions of amino acids are well known and may be made generally without altering the biological activity of the resulting binding domain-immunoglobulin fusion protein molecule. For example, such substitutions are generally made by interchanging within the groups of polar residues, charged residues, hydrophobic residues, small residues, and the like. If necessary, such substitutions may be determined empirically merely by testing the resulting modified protein for the ability to bind to the appropriate cell surface receptors in *in vitro* biological assays, or to bind to appropriate antigens or desired target molecules.

The present invention further relates to nucleic acids which hybridize to constructs of the invention, including for example, binding domain-immunoglobulin fusion protein encoding polynucleotide sequences as provided herein, or their complements, as will be readily apparent to those familiar with the art, if there is at least about 70%, preferably at least about 80-85%, more preferably at least about 90%, and still more preferably at least about 95%, 96%, 97%, 98% or 99% identity between the sequences.

The present invention particularly relates to nucleic acids that hybridize under stringent conditions to, for example, the binding domain-immunoglobulin fusion encoding nucleic acids referred to herein. As used herein, to "hybridize" under conditions of a specified stringency is used to describe the stability of hybrids formed between two single-stranded nucleic acid molecules. Stringency of hybridization is typically expressed in conditions of ionic strength and temperature at which such hybrids are annealed and washed. The term "stringent conditions" refers to conditions that permit hybridization between polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent (*e.g.*, formamide), temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of organic solvents (*e.g.*, formamide), or raising the hybridization temperature. For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, *e.g.*, formamide, while high stringency hybridization can be obtained in

the presence of an organic solvent (*e.g.*, at least about 35% formamide, most preferably at least about 50% formamide). Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, for example, hybridization time, the concentration of detergent, *e.g.*, sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed, and are within the skill in the art. Other typical "high", "medium" and "low" stringency encompass the following conditions or equivalent conditions thereto: high stringency: 0.1 x SSPE or SSC, 0.1% SDS, 65°C; medium stringency: 0.2 x SSPE or SSC, 0.1% SDS, 50°C; and low stringency: 1.0 x SSPE or SSC, 0.1% SDS, 50°C. As known to those having ordinary skill in the art, variations in stringency of hybridization conditions may be achieved by altering the time, temperature and/or concentration of the solutions used for prehybridization, hybridization and wash steps, and suitable conditions may also depend in part on the particular nucleotide sequences of the probe used, and of the blotted, proband nucleic acid sample. Accordingly, it will be appreciated that suitably stringent conditions can be readily selected without undue experimentation where a desired selectivity of the probe is identified, based on its ability to hybridize to one or more certain proband sequences while not hybridizing to certain other proband sequences.

As used herein, preferred "stringent conditions" generally refer to hybridization that will occur only if there is at least about 90-95% and more preferably at least about 97% identity between the sequences. The nucleic acid constructs which hybridize to, for example, binding domain-immunoglobulin fusion encoding nucleic acids referred to herein, in preferred embodiments, encode polypeptides which retain substantially the same biological function or activity as, for example, the binding domain-immunoglobulin fusion polypeptides encoded by the cDNAs.

The nucleic acids of the present invention, also referred to herein as polynucleotides, may be in the form of RNA, for example, mRNA, or in the form of DNA, which DNA includes cDNA (also called "complementary DNA", which is a DNA molecule that is complementary to a specific messenger RNA), genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. A coding sequence

which encodes a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide for use according to the invention may contain portions that are identical to the coding sequence known in the art or described herein for portions thereof, or may be a different coding sequence, which, as a result of the
5 redundancy or degeneracy of the genetic code, encodes the same construct or portion thereof, including all or a portion of a binding domain-immunoglobulin fusion polypeptide.

The nucleic acids which encode constructs of the invention, for example, binding domain-immunoglobulin fusion polypeptides, for use according to the invention may include, but are not limited to: only the coding sequence for the construct, such as a
10 binding domain-immunoglobulin fusion polypeptide; the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide and additional coding sequence; the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequences 5' and/or 3' of the coding
15 sequence for the binding domain-immunoglobulin fusion polypeptide or a portion(s) thereof, which for example may further include but need not be limited to one or more regulatory nucleic acid sequences that may be a regulated or regulatable promoter, enhancer, other transcription regulatory sequence, repressor binding sequence, translation
20 regulatory sequence or any other regulatory nucleic acid sequence. Thus, the term "nucleic acid encoding" or "polynucleotide encoding" a construct, for example, a binding domain-immunoglobulin fusion protein, encompasses a nucleic acid which includes only coding sequence for, for example, a binding domain-immunoglobulin fusion polypeptide as well as a nucleic acid which includes additional coding and/or non-coding sequence(s).

Nucleic acids and oligonucleotides for use as described herein can be
25 synthesized by any method known to those of skill in this art (*see, e.g.*, WO 93/01286, U.S. Application Serial No. 07/723,454; U.S. Patent No. 5,218,088; U.S. Patent No. 5,175,269; U.S. Patent No. 5,109,124). Identification of various oligonucleotides and nucleic acid sequences also involves methods known in the art. For example, the desirable properties, lengths and other characteristics of oligonucleotides useful for cloning are well known. In
30 certain embodiments, synthetic oligonucleotides and nucleic acid sequences may be designed that resist degradation by endogenous host cell nucleolytic enzymes by containing such linkages as: phosphorothioate, methylphosphonate, sulfone, sulfate, ketyl,

phosphorodithioate, phosphoramidate, phosphate esters, and other such linkages that have proven useful in antisense applications. See, e.g., Agrwal *et al.*, *Tetrahedron Lett.* 28:3539-3542 (1987); Miller *et al.*, *J. Am. Chem. Soc.* 93:6657-6665 (1971); Stec *et al.*, *Tetrahedron Lett.* 26:2191-2194 (1985); Moody *et al.*, *Nucl. Acids Res.* 12:4769-4782 (1989); Uznanski *et al.*, *Nucl. Acids Res.* (1989); Letsinger *et al.*, *Tetrahedron* 40:137-143 (1984); Eckstein, *Annu. Rev. Biochem.* 54:367-402 (1985); Eckstein, *Trends Biol. Sci.* 14:97-100 (1989); Stein In: *Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression*, Cohen, Ed, Macmillan Press, London, pp. 97-117 (1989); Jager *et al.*, *Biochemistry* 27:7237-7246 (1988).

10 In one embodiment, the present invention provides truncated components (e.g., binding domain polypeptide, hinge region polypeptide, linker, *etc.*) for use in a construct of the invention, for example, a binding domain-immunoglobulin fusion protein. In another embodiment the invention provides nucleic acids encoding a construct of the invention, for example, a binding domain-immunoglobulin fusion protein having such
15 truncated components. A truncated molecule may be any molecule that comprises less than a full length version of the molecule of interest. Truncated molecules provided by the present invention may include truncated biological polymers, and in preferred embodiments of the invention such truncated molecules may be truncated nucleic acid molecules or truncated polypeptides. Truncated nucleic acid molecules have less than the
20 full length nucleotide sequence of a known or described nucleic acid molecule, where such a known or described nucleic acid molecule may be a naturally occurring, a synthetic, or a recombinant nucleic acid molecule, so long as one skilled in the art would regard it as a full length molecule. Thus, for example, truncated nucleic acid molecules that correspond to a gene sequence contain less than the full length gene where the gene comprises coding and
25 non-coding sequences, promoters, enhancers and other regulatory sequences, flanking sequences and the like, and other functional and non-functional sequences that are recognized as part of the gene. In another example, truncated nucleic acid molecules that correspond to a mRNA sequence contain less than the full length mRNA transcript, which may include various translated and non-translated regions as well as other functional and
30 non-functional sequences.

In other preferred embodiments, truncated molecules are polypeptides that comprise less than the full-length amino acid sequence of a particular protein or

polypeptide component. As used herein "deletion" has its common meaning as understood by those familiar with the art, and may refer to molecules that lack one or more portions of a sequence from either terminus or from a non-terminal region, relative to a corresponding full length molecule, for example, as in the case of truncated molecules provided herein.

5 Truncated molecules that are linear biological polymers such as nucleic acid molecules or polypeptides may have one or more of a deletion from either terminus of the molecule and/or one or more deletions from a non-terminal region of the molecule, where such deletions may be deletions of from about 1-1500 contiguous nucleotide or amino acid residues, preferably about 1-500 contiguous nucleotide or amino acid residues and more
10 preferably about 1-300 contiguous nucleotide or amino acid residues, including deletions of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31-40, 41-50, 51-74, 75-100, 101-150, 151-200, 201-250 or 251-299 contiguous nucleotide or amino acid residues. In certain particularly preferred embodiments truncated nucleic acid molecules may have a deletion of about 270-330
15 contiguous nucleotides. In certain more preferred embodiments, truncated polypeptide molecules may have a deletion, for example, of about 80-140 contiguous amino acids.

The present invention further relates to variants of the herein referenced nucleic acids that encode fragments, analogs and/or derivatives of a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide. The
20 variants of the nucleic acids encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, may be naturally occurring allelic variants of one or more portions of the the nucleic acid sequences included therein, or non-naturally occurring variants of such sequences or portions or sequences, including sequences varied by molecular engineering using, for example, methods known in the art for varying
25 sequence. As is known in the art, an allelic variant is an alternate form of a nucleic acid sequence which may have at least one of a substitution, a deletion or an addition of one or more nucleotides, any of which does not substantially or undesireably alter the function of the encoded binding domain-immunoglobulin fusion polypeptide.

Variants and derivatives of constructs of the invention, for example, binding
30 domain-immunoglobulin fusion proteins, may be obtained by mutations of nucleotide sequences encoding, for example, binding domain-immunoglobulin fusion polypeptides or any portion thereof. Alterations of the native amino acid sequence may be accomplished

by any of a number of conventional methods. Mutations can be introduced at particular loci, for example, by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, for example, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene wherein predetermined codons can be altered by substitution, deletion or insertion. Exemplary methods of making such alterations are disclosed by Walder *et al.*, 1986 *Gene* 42:133; Bauer *et al.*, 1985 *Gene* 37:73; Craik, January 1985 *BioTechniques* 12-19; Smith *et al.*, January 1985 *Genetic Engineering: Principles and Methods BioTechniques* 12-19; Costa GL, *et al.*, "Site-directed mutagenesis using a rapid PCR-based method," 1996 *Methods Mol Biol.* 57:239-48; Rashtchian A., "Novel methods for cloning and engineering genes using the polymerase chain reaction," 1995 *Curr Opin Biotechnol.* 6(1):30-6; Sharon J, *et al.*, "Oligonucleotide-directed mutagenesis of antibody combining sites," 1993 *Int Rev Immunol.* 10(2-3):113-27; Kunkel, 1985 *Proc. Natl. Acad. Sci. USA* 82:488; Kunkel *et al.*, 1987 *Methods in Enzymol.* 154:367; and, U.S. Patent Nos. 4,518,584 and 4,737,462.

As an example, modification of DNA may be performed by site-directed mutagenesis of DNA encoding a protein combined with the use of DNA amplification methods using primers to introduce and amplify alterations in the DNA template, such as PCR splicing by overlap extension (SOE). Site-directed mutagenesis is typically effected using a phage vector that has single- and double-stranded forms, such as M13 phage vectors, which are well known and commercially available. Other suitable vectors that contain a single-stranded phage origin of replication may be used. See, *e.g.*, Veira *et al.*, 1987 *Meth. Enzymol.* 15:3. In general, site-directed mutagenesis is performed by preparing a single-stranded vector that encodes the protein of interest (*e.g.*, all or a component portion of a given binding domain-immunoglobulin fusion protein). An oligonucleotide primer that contains the desired mutation within a region of homology to the DNA in the single-stranded vector is annealed to the vector followed by addition of a DNA polymerase, such as *E. coli* DNA polymerase I (Klenow fragment), which uses the double stranded region as a primer to produce a heteroduplex in which one strand encodes the altered sequence and the other the original sequence. The heteroduplex is introduced into

appropriate bacterial cells and clones that include the desired mutation are selected. The resulting altered DNA molecules may be expressed recombinantly in appropriate host cells to produce the modified protein.

Equivalent DNA constructs that include code for additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences not needed or desired for biological activity, for example, are also encompassed by the invention. For example, and as discussed above, sequences encoding Cys residues that are not desirable or essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, for example, thus preventing formation of incorrect or undesired intramolecular disulfide bridges upon synthesis or renaturation.

A "host cell" or "recombinant host cell" is a cell that contains a vector, *e.g.*, an expression vector, or a cell that has otherwise been manipulated by recombinant techniques to express a protein of interest. Host organisms include those organisms in which recombinant production of constructs of the invention, for example, binding domain-immunoglobulin fusion products encoded by the recombinant constructs of the present invention may occur, such as bacteria (for example, *E. coli*), yeast (for example, *Saccharomyces cerevisiae* and *Pichia pastoris*), insect cells, and mammalian cells, including *in vitro* and *in vivo* expression. Host organisms thus may include organisms for the construction, propagation, expression or other steps in the production of the compositions provided herein. Hosts include subjects in which immune responses take place, as described herein. Presently preferred host organisms for production of constructs of the invention that produce glycosylated proteins are mammalian cells or other cell systems that permit the expression and recovery of glycosylated proteins. Other cell lines include inbred murine strains and murine cell lines, and human cells and cell lines.

A DNA construct encoding a desired construct of the invention, for example, a binding domain-immunoglobulin fusion protein is introduced into a vector, for example, a plasmid, for expression in an appropriate host. In preferred embodiments, the host is a mammalian host, for example, a mammalian cell line. The sequence encoding the ligand or nucleic acid binding domain is preferably codon-optimized for expression in the particular host. Thus, for example, if a construct, for example, is a human binding domain-immunoglobulin fusion and is expressed in bacteria, the codons may be optimized for

bacterial usage. For small coding regions, the gene can be synthesized as a single oligonucleotide. For larger proteins, splicing of multiple oligonucleotides, mutagenesis, or other techniques known to those in the art may be used. The sequences of nucleotides in plasmids or other vectors that are regulatory regions, such as promoters and operators, are operationally associated with one another for transcription. The sequence of nucleotides encoding a binding domain-immunoglobulin fusion protein may also include DNA encoding a secretion signal, whereby the resulting peptide is a precursor protein. The resulting processed protein may be recovered from the periplasmic space or the fermentation medium.

In preferred embodiments, the DNA plasmids may also include a transcription terminator sequence. As used herein, a "transcription terminator region" is a sequence that signals transcription termination. The entire transcription terminator may be obtained from a protein-encoding gene, which may be the same or different from the inserted binding domain-immunoglobulin fusion encoding gene or the source of the promoter. Transcription terminators are optional components of the expression systems herein, but are employed in preferred embodiments.

The plasmids or other vectors used herein include a promoter in operative association with the DNA encoding the protein or polypeptide of interest and are designed for expression of proteins in a suitable host as described above (*e.g.*, bacterial, murine, or human) depending upon the desired use of the plasmid (*e.g.*, administration of a vaccine containing binding domain-immunoglobulin fusion encoding sequences). Suitable promoters for expression of proteins and polypeptides herein are widely available and are well known in the art. Inducible promoters or constitutive promoters that are linked to regulatory regions are preferred. Such promoters include, for example, but are not limited to, the T7 phage promoter and other T7-like phage promoters, such as the T3, T5 and SP6 promoters, the *trp*, *lpp*, and *lac* promoters, such as the *lacUV5*, from *E. coli*; the P10 or polyhedrin gene promoter of baculovirus/insect cell expression systems (*see, e.g.*, U.S. Patent Nos. 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784) and inducible promoters from other eukaryotic expression systems. For expression of the proteins such promoters are inserted in a plasmid in operative linkage with a control region such as the *lac* operon.

Preferred promoter regions are those that are inducible and functional in mammalian cells, for example. Examples of suitable inducible promoters and promoter regions for bacterial expression include, but are not limited to: the *E. coli* lac operator responsive to isopropyl β -D-thiogalactopyranoside (IPTG; see Nakamura *et al.*, 1979 *Cell* 5 18:1109-1117); the metallothionein promoter metal-regulatory-elements responsive to heavy-metal (*e.g.*, zinc) induction (see, *e.g.*, U.S. Patent No. 4,870,009); the phage T7lac promoter responsive to IPTG (see, *e.g.*, U.S. Patent No. 4,952,496; and Studier *et al.*, 1990 *Meth. Enzymol.* 185:60-89) and the TAC promoter. Depending on the expression host system to be used, plasmids may optionally include a selectable marker gene or genes that are functional in the host. Thus, for example, a selectable marker gene includes any gene that confers a phenotype on bacteria that allows transformed bacterial cells to be identified and selectively grown from among a vast majority of untransformed cells. Suitable selectable marker genes for bacterial hosts, for example, include the ampicillin resistance gene (Amp^r), tetracycline resistance gene (Tc^r) and the kanamycin resistance gene (Kan^r). 10 The kanamycin resistance gene is presently preferred for bacterial expression.

In various expression systems, plasmids or other vectors may also include DNA encoding a signal for secretion of the operably linked protein. Secretion signals suitable for use are widely available and are well known in the art. Prokaryotic and eukaryotic secretion signals functional in *E. coli* may be employed. Depending on the expression systems, presently preferred secretion signals may include, but are not limited to, those encoded by the following *E. coli* genes: ompA, ompT, ompF, ompC, beta-lactamase, and alkaline phosphatase, and the like (von Heijne, *J. Mol. Biol.* 184:99-105, 1985). In addition, the bacterial pelB gene secretion signal (Lei *et al.*, *J. Bacteriol.* 169:4379, 1987), the phoA secretion signal, and the cek2 functional in insect cell may be employed. The most preferred secretion signal for certain expression systems is the *E. coli* ompA secretion signal. Other prokaryotic and eukaryotic secretion signals known to those of skill in the art may also be employed (*see, e.g.*, von Heijne, *J. Mol. Biol.* 184:99-105, 1985). Using the methods described herein, one of skill in the art can substitute secretion signals that are functional, for example, in yeast, insect or mammalian cells to secrete 20 proteins from those cells.

Preferred plasmids for transformation of *E. coli* cells include the pET expression vectors (*e.g.*, pET-11a, pET-12a-c, pET-15b; *see* U.S. Patent No. 4,952,496;

available from Novagen, Madison, WI.). Other preferred plasmids include the pKK plasmids, particularly pKK 223-3, which contains the tac promoter (Brosius *et al.*, 1984 *Proc. Natl. Acad. Sci.* 81:6929; Ausubel *et al.*, *Current Protocols in Molecular Biology*; U.S. Patent Nos. 5,122,463, 5,173,403, 5,187,153, 5,204,254, 5,212,058, 5,212,286, 5,215,907, 5,220,013, 5,223,483, and 5,229,279). Plasmid pKK has been modified by replacement of the ampicillin resistance gene with a kanamycin resistance gene. (Available from Pharmacia; obtained from pUC4K, see, *e.g.*, Vieira *et al.* (1982 *Gene* 19:259-268; and U.S. Patent No. 4,719,179.) Baculovirus vectors, such as pBlueBac (also called pJVETL and derivatives thereof), particularly pBlueBac III (see, *e.g.*, U.S. Patent Nos. 5,278,050, 5,244,805, 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784; available from Invitrogen, San Diego) may also be used for expression of the polypeptides in insect cells. Other plasmids include the pIN-IIIompA plasmids (see U.S. Patent No. 4,575,013; see also Duffaud *et al.*, *Meth. Enz.* 153:492-507, 1987), such as pIN-IIIompA2.

Preferably, if one or more DNA molecules is replicated in bacterial cells, the preferred host is *E. coli*. The preferred DNA molecule is such a system also includes a bacterial origin of replication, to ensure the maintenance of the DNA molecule from generation to generation of the bacteria. In this way, large quantities of the DNA molecule can be produced by replication in bacteria. In such expression systems, preferred bacterial origins of replication include, but are not limited to, the fl-ori and col E1 origins of replication. Preferred hosts for such systems contain chromosomal copies of DNA encoding T7 RNA polymerase operably linked to an inducible promoter, such as the lacUV promoter (see U.S. Patent No. 4,952,496). Such hosts include, but are not limited to, lysogens *E. coli* strains HMS174(DE3)pLysS, BL21(DE3)pLysS, HMS174(DE3) and BL21(DE3). Strain BL21(DE3) is preferred. The pLys strains provide low levels of T7 lysozyme, a natural inhibitor of T7 RNA polymerase.

The DNA molecules provided may also contain a gene coding for a repressor protein. The repressor protein is capable of repressing the transcription of a promoter that contains sequences of nucleotides to which the repressor protein binds. The promoter can be derepressed by altering the physiological conditions of the cell. For example, the alteration can be accomplished by adding to the growth medium a molecule that inhibits the ability to interact with the operator or with regulatory proteins or other regions of the DNA or by altering the temperature of the growth media. Preferred

repressor proteins include, but are not limited to the *E. coli* lacI repressor responsive to IPTG induction, the temperature sensitive λ cI857 repressor, and the like. The *E. coli* lacI repressor is preferred.

In general, recombinant constructs of the subject invention will also contain elements necessary for transcription and translation. In particular, such elements are preferred where the recombinant expression construct containing nucleic acid sequences encoding binding domain-immunoglobulin fusion proteins is intended for expression in a host cell or organism. In certain embodiments of the present invention, cell type preferred or cell type specific expression of a cell binding domain-immunoglobulin fusion encoding gene may be achieved by placing the gene under regulation of a promoter. The choice of the promoter will depend upon the cell type to be transformed and the degree or type of control desired. Promoters can be constitutive or active and may further be cell type specific, tissue specific, individual cell specific, event specific, temporally specific or inducible. Cell-type specific promoters and event type specific promoters are preferred. Examples of constitutive or nonspecific promoters include the SV40 early promoter (U.S. Patent No. 5,118,627), the SV40 late promoter (U.S. Patent No. 5,118,627), CMV early gene promoter (U.S. Patent No. 5,168,062), and adenovirus promoter. In addition to viral promoters, cellular promoters are also amenable within the context of this invention. In particular, cellular promoters for the so-called housekeeping genes are useful. Viral promoters are preferred, because generally they are stronger promoters than cellular promoters. Promoter regions have been identified in the genes of many eukaryotes including higher eukaryotes, such that suitable promoters for use in a particular host can be readily selected by those skilled in the art.

Inducible promoters may also be used. These promoters include MMTV LTR (PCT WO 91/13160), inducible by dexamethasone; metallothionein promoter, inducible by heavy metals; and promoters with cAMP response elements, inducible by cAMP. By using an inducible promoter, the nucleic acid sequence encoding a binding domain-immunoglobulin fusion protein may be delivered to a cell by the subject invention expression construct and will remain quiescent until the addition of the inducer. This allows further control on the timing of production of the gene product.

Event-type specific promoters are active or up regulated only upon the occurrence of an event, such as tumorigenicity or viral infection. The HIV LTR is a well-

known example of an event-specific promoter. The promoter is inactive unless the *tat* gene product is present, which occurs upon viral infection. Some event-type promoters are also tissue-specific.

5 Additionally, promoters that are coordinately regulated with a particular cellular gene may be used. For example, promoters of genes that are coordinately expressed may be used when expression of a particular binding construct of the invention, for example, a binding domain-immunoglobulin fusion protein-encoding gene is desired in concert with expression of one or more additional endogenous or exogenously introduced genes. This type of promoter is especially useful when one knows the pattern of gene
10 expression relevant to induction of an immune response in a particular tissue of the immune system, so that specific immunocompetent cells within that tissue may be activated or otherwise recruited to participate in the immune response.

In addition to the promoter, repressor sequences, negative regulators, or tissue-specific silencers may be inserted to reduce non-specific expression of binding
15 domain-immunoglobulin fusion protein encoding genes in certain situations, such as, for example, a host that is transiently immunocompromised as part of a therapeutic strategy. Multiple repressor elements may be inserted in the promoter region. Repression of transcription is independent on the orientation of repressor elements or distance from the promoter. One type of repressor sequence is an insulator sequence. Such sequences inhibit
20 transcription (Dunaway *et al.*, 1997 *Mol Cell Biol* 17: 182-9; Gdula *et al.*, 1996 *Proc Natl Acad Sci USA* 93:9378-83, Chan *et al.*, 1996 *J Virol* 70: 5312-28; Scott and Geyer, 1995 *EMBO J* 14:6258-67; Kalos and Fournier, 1995 *Mol Cell Biol* 15:198-207; Chung *et al.*, 1993 *Cell* 74: 505-14) and will silence undesired background transcription.

Repressor elements have also been identified in the promoter regions of the
25 genes for type II (cartilage) collagen, choline acetyltransferase, albumin (Hu *et al.*, 1992 *J. Cell Growth Differ.* 3(9):577-588), phosphoglycerate kinase (PGK-2) (Misuno *et al.*, 1992 *Gene* 119(2):293-297), and in the 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase gene. (Lemaigre *et al.*, *Mol. Cell Biol.* 11(2):1099-1106). Furthermore, the negative regulatory element Tse-1 has been identified in a number of liver specific genes, and has
30 been shown to block cAMP response element(CRE)-mediated induction of gene activation in hepatocytes. (Boshart *et al.*, 1990 *Cell* 61(5):905-916.).

In preferred embodiments, elements that increase the expression of the desired product are incorporated into the construct. Such elements include internal ribosome binding sites (IRES; Wang and Siddiqui, 1995 *Curr. Top. Microbiol. Immunol.* 203:99; Ehrenfeld and Semler, 1995 *Curr. Top. Microbiol. Immunol.* 203:65; Rees *et al.*, 1996 *Biotechniques* 20:102; Sugimoto *et al.*, 1994 *Biotechnology* 12:694). IRES increase translation efficiency. As well, other sequences may enhance expression. For some genes, sequences especially at the 5' end inhibit transcription and/or translation. These sequences are usually palindromes that can form hairpin structures. Any such sequences in the nucleic acid to be delivered are generally deleted. Expression levels of the transcript or translated product are assayed to confirm or ascertain which sequences affect expression. Transcript levels may be assayed by any known method, including Northern blot hybridization, RNase probe protection and the like. Protein levels may be assayed by any known method, including ELISA, western blot, immunocytochemistry or other well-known techniques.

Other elements may be incorporated into the constructs of the invention, for example, into binding domain-immunoglobulin fusion protein encoding constructs of the present invention. In preferred embodiments, the construct includes a transcription terminator sequence, including a polyadenylation sequence, splice donor and acceptor sites, and an enhancer. Other elements useful for expression and maintenance of the construct in mammalian cells or other eukaryotic cells may also be incorporated (*e.g.*, origin of replication). Because the constructs are conveniently produced in bacterial cells, elements that are necessary for, or that enhance, propagation in bacteria are incorporated. Such elements include an origin of replication, a selectable marker and the like.

As provided herein, an additional level of controlling the expression of nucleic acids encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, delivered to cells for gene therapy, for example, may be provided by simultaneously delivering two or more differentially regulated nucleic acid constructs. The use of such a multiple nucleic acid construct approach may permit coordinated regulation of an immune response such as, for example, spatiotemporal coordination that depends on the cell type and/or presence of another expressed encoded component. Those familiar with the art will appreciate that multiple levels of regulated gene expression may be achieved in a similar manner by selection of suitable regulatory

sequences, including but not limited to promoters, enhancers and other well known gene regulatory elements.

The present invention also relates to vectors, and to constructs prepared from known vectors that include nucleic acids of the present invention, and in particular to
5 "recombinant expression constructs", including any of various known constructs, including delivery constructs, useful for gene therapy, that include any nucleic acids encoding, for example, binding domain-immunoglobulin fusion proteins and polypeptides according to the invention as provided herein; to host cells which are genetically engineered with vectors and/or other constructs of the invention and to methods of administering expression
10 or other constructs comprising nucleic acid sequences encoding, for example, binding domain-immunoglobulin fusion polypeptides and fusion proteins of the invention, or fragments or variants thereof, by recombinant techniques.

Various constructs of the invention, including for example, binding domain-immunoglobulin fusion proteins, can be expressed in virtually any host cell, including *in*
15 *vivo* host cells in the case of use for gene therapy, under the control of appropriate promoters, depending on the nature of the construct (*e.g.*, type of promoter, as described above), and on the nature of the desired host cell (*e.g.*, whether postmitotic terminally differentiated or actively dividing; *e.g.*, whether the expression construct occurs in host cell as an episome or is integrated into host cell genome).

20 Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described, for example, by Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, NY, (1989); as noted herein, in particularly preferred embodiments of the invention, recombinant expression is conducted in mammalian cells that have been transfected or transformed with the subject invention
25 recombinant expression construct. See also, for example, Machida, CA., "Viral Vectors for Gene Therapy: Methods and Protocols"; Wolff, JA, "Gene Therapeutics: Methods and Applications of Direct Gene Transfer" (Birkhauser 1994); Stein, U and Walther, W (eds.P, "Gene Therapy of Cancer: Methods and Protocols" (Humana Press 2000); Robbins, PD (ed.), "Gene Therapy Protocols" (Humana Press 1997); Morgan, JR (ed.), "Gene Therapy
30 Protocols" (Humana Press 2002); Meager, A (ed.), "Gene Therapy Technologies, Applications and Regulations: From Laboratory to Clinic" (John Wiley & Sons Inc. 1999); MacHida, CA and Constant, JG, "Viral Vectors for Gene Therapy: Methods and Protocols"

(Humana Press 2002); "New Methods Of Gene Therapy For Genetic Metabolic Diseases NIH Guide," Volume 22, Number 35, October 1, 1993. See also recent U.S. patents relating to gene therapy, including vaccines, which include U.S. Pat. Nos. 6,384,210 ("Solvent for biopolymer synthesis, solvent microdroplets and methods of use"); 6,384,203 ("Family of immunoregulators designated leukocyte immunoglobulin-like receptors (LIR)"); 6,384,202 ("Cell-specific active compounds regulated by the cell cycle"); 6,384,018 ("Polynucleotide tuberculosis vaccine"); 6,383,814 ("Cationic amphiphiles for intracellular delivery of therapeutic molecules"); 6,383,811 ("Polyampholytes for delivering polyions to a cell"); 6,383,795 ("Efficient purification of adenovirus"); 6,383,794 ("Methods of producing high titer recombinant adeno-associated virus"); 6,383,785 ("Self-enhancing, pharmacologically controllable expression systems"); 6,383,753 ("Yeast mammalian regulators of cell proliferation"); 6,383,746 ("Functional promoter for CCR5"); 6,383,743 ("Method for serial analysis of gene expression"); 6,383,738 ("Herpes simplex virus ORF P is a repressor of viral protein synthesis"); 6,383,737 ("Human oxalyl-CoA Decarboxylase"); 6,383,733 ("Methods of screening for pharmacologically active compounds for the treatment of tumour diseases"); 6,383,522 ("Toxicity reduced composition containing an anti-neoplastic agent and a shark cartilage extract"); 6,383,512 ("Vesicular complexes and methods of making and using the same"); 6,383,481 ("Method for transplantation of hemopoietic stem cells"); 6,383,478 ("Polymeric encapsulation system promoting angiogenesis"); 6,383,138 ("Method for transdermal sampling of analytes"); 6,380,382 ("Gene encoding a protein having diagnostic, preventive, therapeutic, and other uses"); 6,380,371 ("Endoglycan: a novel protein having selectin ligand and chemokine presentation activity"); 6,380,369 ("Human DNA mismatch repair proteins"); 6,380,362 ("Polynucleotides, polypeptides expressed by the polynucleotides and methods for their use"); 6,380,170 ("Nucleic acid construct for the cell cycle regulated expression of structural genes"); 6,380,169 ("Metal complex containing oligonucleoside cleavage compounds and therapies"); 6,379,967 ("Herpesvirus saimiri as viral vector"); 6,379,966 ("Intravascular delivery of non-viral nucleic acid protease proteins, and uses thereof").

Typically, for example, expression constructs are derived from plasmid vectors. One preferred construct is a modified pNASS vector (Clontech, Palo Alto, CA), which has nucleic acid sequences encoding an ampicillin resistance gene, a

polyadenylation signal and a T7 promoter site. Other suitable mammalian expression vectors are well known (*see, e.g.,* Ausubel *et al.*, 1995; Sambrook *et al., supra*; *see also, e.g.,* catalogues from Invitrogen, San Diego, CA; Novagen, Madison, WI; Pharmacia, Piscataway, NJ; and others). Presently preferred constructs may be prepared that include a
5 dihydrofolate reductase (DHFR) encoding sequence under suitable regulatory control, for promoting enhanced production levels of the binding domain-immunoglobulin fusion protein, which levels result from gene amplification following application of an appropriate selection agent (*e.g.,* methotrexate).

Generally, recombinant expression vectors will include origins of
10 replication and selectable markers permitting transformation of the host cell, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, as described above. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences. Thus, for example, the binding domain-immunoglobulin fusion protein encoding nucleic
15 acids as provided herein may be included in any one of a variety of expression vector constructs as a recombinant expression construct for expressing a binding domain-immunoglobulin fusion polypeptide in a host cell. In certain preferred embodiments the constructs are included in formulations that are administered *in vivo*. Such vectors and
20 constructs include chromosomal, nonchromosomal and synthetic DNA sequences, *e.g.,* derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA, such as vaccinia, adenovirus, fowl pox virus, and pseudorabies, or replication deficient retroviruses as described below. However, any other vector may be used for preparation of a recombinant expression construct, and in preferred embodiments such a vector will be replicable and viable in the
25 host.

The appropriate DNA sequence(s) may be inserted into a vector, for example, by a variety of procedures. In general, a DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic
30 reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described, for example, in Ausubel

et al. (1993 *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, MA); Sambrook *et al.* (1989 *Molecular Cloning*, Second Ed., Cold Spring Harbor Laboratory, Plainview, NY); Maniatis *et al.* (1982 *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, NY); Glover (Ed.) (1985 *DNA Cloning Vol. I and II*, IRL Press, Oxford, UK); Hames and Higgins (Eds.), (1985 *Nucleic Acid Hybridization*, IRL Press, Oxford, UK); and elsewhere.

The DNA sequence in the expression vector is operatively linked to at least one appropriate expression control sequence(s) (*e.g.*, a constitutive promoter or a regulated promoter) to direct mRNA synthesis. Representative examples of such expression control sequences include promoters of eukaryotic cells or their viruses, as described above. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art, and preparation of certain particularly preferred recombinant expression constructs comprising at least one promoter or regulated promoter operably linked to a nucleic acid encoding an binding domain-immunoglobulin fusion polypeptide is described herein.

Transcription of the DNA encoding proteins and polypeptides included within the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are *cis*-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Gene therapy is the use of genetic material to treat disease. It comprises strategies to replace defective genes or add new genes to cells and/or tissues, and is being developed for application in the treatment of cancer, the correction of metabolic disorders and in the field of immunotherapy. Gene therapies of the invention include the use of various constructs of the invention, with or without a separate carrier or delivery vehicle or constructs, for treatment of the diseases, disorders, and/or conditions noted herein. Such constructs may also be used as vaccines for treatment or prevention of the diseases,

disorders, and/or conditions noted herein. DNA vaccines, for example, make use of polynucleotides encoding immunogenic protein and nucleic acid determinants to stimulate the immune system against pathogens or tumor cells. Such strategies can stimulate either acquired or innate immunity or can involve the modification of immune function through cytokine expression. *In vivo* gene therapy involves the direct injection of genetic material into a patient or animal model of human disease. Vaccines and immune modulation are systemic therapies. With tissue-specific *in vivo* therapies, such as those that aim to treat cancer, localized gene delivery and/or expression/targeting systems are preferred. Diverse gene therapy vectors have been designed to target specific tissues, and procedures have been developed to physically target specific tissues, for example, using catheter-based technologies, all of which are contemplated herein. *Ex vivo* approaches to gene therapy are also contemplated herein and involve the removal, genetic modification, expansion and re-administration of a patient's own cells. Examples include bone marrow transplantation for cancer treatment or the genetic modification of lymphoid progenitor cells. *Ex vivo* gene therapy is preferably applied to the treatment of cells that are easily accessible and can survive in culture during the gene transfer process (such as blood or skin cells).

Useful gene therapy vectors include adenoviral vectors, lentiviral vectors, Adeno-associated virus (AAV) vectors, Herpes Simplex Virus (Hsv) vectors, and retroviral vectors. Gene therapies may also be carried out using "naked DNA," liposome-based delivery, lipid-based delivery (including DNA attached to positively charged lipids), and electroporation.

As provided herein, in certain embodiments, including but not limited to gene therapy embodiments, the vector may be a viral vector such as, for example, a retroviral vector. Miller *et al.*, 1989 *BioTechniques* 7:980; Coffin and Varmus, 1996 *Retroviruses*, Cold Spring Harbor Laboratory Press, NY. For example, retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

Retroviruses are RNA viruses that can replicate and integrate into the genome of a host cell via a DNA intermediate. This DNA intermediate, or provirus, may

be stably integrated into the host cell DNA. According to certain embodiments of the present invention, an expression construct may comprise a retrovirus into which a foreign gene that encodes a foreign protein is incorporated in place of normal retroviral RNA. When retroviral RNA enters a host cell coincident with infection, the foreign gene is also
5 introduced into the cell, and may then be integrated into host cell DNA as if it were part of the retroviral genome. Expression of this foreign gene within the host results in expression of the foreign protein.

Most retroviral vector systems that have been developed for gene therapy are based on murine retroviruses. Such retroviruses exist in two forms, as free viral
10 particles referred to as virions, or as proviruses integrated into host cell DNA. The virion form of the virus contains the structural and enzymatic proteins of the retrovirus (including the enzyme reverse transcriptase), two RNA copies of the viral genome, and portions of the source cell plasma membrane containing viral envelope glycoprotein. The retroviral genome is organized into four main regions: the Long Terminal Repeat (LTR), which
15 contains *cis*-acting elements necessary for the initiation and termination of transcription and is situated both 5' and 3' of the coding genes, and the three coding genes *gag*, *pol*, and *env*. These three genes *gag*, *pol*, and *env* encode, respectively, internal viral structures, enzymatic proteins (such as integrase), and the envelope glycoprotein (designated gp70 and p15e) which confers infectivity and host range specificity of the virus, as well as the "R"
20 peptide of undetermined function.

Separate packaging cell lines and vector producing cell lines have been developed because of safety concerns regarding the uses of retroviruses, including their use in expression constructs as provided by the present invention. Briefly, this methodology employs the use of two components, a retroviral vector and a packaging cell line (PCL).
25 The retroviral vector contains long terminal repeats (LTRs), the foreign DNA to be transferred and a packaging sequence (*y*). This retroviral vector will not reproduce by itself because the genes that encode structural and envelope proteins are not included within the vector genome. The PCL contains genes encoding the *gag*, *pol*, and *env* proteins, but does not contain the packaging signal "y". Thus, a PCL can only form empty
30 virion particles by itself. Within this general method, the retroviral vector is introduced into the PCL, thereby creating a vector-producing cell line (VCL). This VCL

manufactures virion particles containing only the retroviral vector's (foreign) genome, and therefore has previously been considered to be a safe retrovirus vector for therapeutic use.

“Retroviral vector construct” refers to an assembly that is, within preferred embodiments of the invention, capable of directing the expression of a sequence(s) or gene(s) of interest, such as binding domain-immunoglobulin fusion encoding nucleic acid sequences. Briefly, the retroviral vector construct must include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis and a 3' LTR. A wide variety of heterologous sequences may be included within the vector construct, including for example, sequences which encode a protein (*e.g.*, cytotoxic protein, disease-associated antigen, immune accessory molecule, or replacement gene), or which are useful as a molecule itself (*e.g.*, as a ribozyme or antisense sequence).

Retroviral vector constructs of the present invention may be readily constructed from a wide variety of retroviruses, including for example, B, C, and D type retroviruses as well as spumaviruses and lentiviruses (see, *e.g.*, RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985). Such retroviruses may be readily obtained from depositories or collections such as the American Type Culture Collection (“ATCC”; Rockville, Maryland), or isolated from known sources using commonly available techniques. Any of the above retroviruses may be readily utilized in order to assemble or construct retroviral vector constructs, packaging cells, or producer cells of the present invention given the disclosure provided herein, and standard recombinant techniques (*e.g.*, Sambrook *et al*, *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, 1989; Kunkle, 1985 *PNAS* 82:488).

Suitable promoters for use in viral vectors generally may include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, *et al.*, 1989 *Biotechniques* 7:980-990, or any other promoter (*e.g.*, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β -actin promoters). Other viral promoters that may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein, and may be from among either regulated promoters or promoters as described above.

As described above, the retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, ψ -2, ψ -AM, PA12, T19-14X, VT-19-17-H2, ψ CRE, ψ CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, *Human Gene Therapy*, 1:5-14 (1990). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO_4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles that include the nucleic acid sequence(s) encoding the binding domain-immunoglobulin fusion polypeptides or fusion proteins. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the binding domain-immunoglobulin fusion polypeptide or fusion protein. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, circulating peripheral blood mononuclear and polymorphonuclear cells including myelomonocytic cells, lymphocytes, myoblasts, tissue macrophages, dendritic cells, Kupffer cells, lymphoid and reticuloendothelia cells of the lymph nodes and spleen, keratinocytes, endothelial cells, and bronchial epithelial cells.

As another example of an embodiment of the invention in which a viral vector is used to prepare, for example, a recombinant binding domain-immunoglobulin fusion expression construct, in one preferred embodiment, host cells transduced by a recombinant viral construct directing the expression of binding domain-immunoglobulin fusion polypeptides or fusion proteins may produce viral particles containing expressed binding domain-immunoglobulin fusion polypeptides or fusion proteins that are derived from portions of a host cell membrane incorporated by the viral particles during viral budding.

In another aspect, the present invention relates to host cells containing the herein described nucleic acid constructs, such as, for example, recombinant binding domain-immunoglobulin fusion expression constructs. Host cells are genetically engineered (transduced, transformed or transfected) with the vectors and/or expression

constructs of this invention that may be, for example, a cloning vector, a shuttle vector, or an expression construct. The vector or construct may be, for example, in the form of a plasmid, a viral particle, a phage, *etc.* The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying particular genes such as genes encoding binding domain-immunoglobulin fusion polypeptides or binding domain-immunoglobulin fusion proteins. The culture conditions for particular host cells selected for expression, such as temperature, pH and the like, will be readily apparent to the ordinarily skilled artisan.

The host cell for production or expression of a construct of the invention, for example, can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Representative examples of appropriate host cells according to the present invention include, but need not be limited to, bacterial cells, such as *E. coli*, *Streptomyces*, *Salmonella typhimurium*; fungal cells, such as yeast; insect cells, such as *Drosophila S2* and *Spodoptera Sf9*; animal cells, such as CHO, COS or 293 cells; adenoviruses; plant cells, or any suitable cell already adapted to *in vitro* propagation or so established *de novo*. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, 1981 *Cell* 23:175, and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences, for example as described herein regarding the preparation of binding domain-immunoglobulin fusion expression constructs. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Introduction of the construct into the host cell can be effected by a variety of methods with which those skilled in the art will be familiar, including but not limited to, for example, calcium phosphate transfection, DEAE-

Dextran mediated transfection, or electroporation (Davis *et al.*, 1986 *Basic Methods in Molecular Biology*).

The present invention constructs, for example, binding domain-immunoglobulin fusion proteins, or compositions comprising one or more polynucleotides encoding same as described herein (for example, to be administered under conditions and for a time sufficient to permit expression of a binding domain-immunoglobulin fusion protein in a host cell *in vivo* or *in vitro*, for gene therapy, for example, among other things), may be formulated into pharmaceutical compositions for administration according to well known methodologies. Pharmaceutical compositions generally comprise one or more recombinant expression constructs, and/or expression products of such constructs, in combination with a pharmaceutically acceptable carrier, excipient or diluent. Such carriers will be nontoxic to recipients at the dosages and concentrations employed. For nucleic acid-based formulations, or for formulations comprising expression products of the subject invention recombinant constructs, about 0.01 µg/kg to about 100 mg/kg body weight will be administered, for example, typically by the intradermal, subcutaneous, intramuscular or intravenous route, or by other routes. A preferred dosage, for example, is about 1 µg/kg to about 1 mg/kg, with about 5 µg/kg to about 200 µg/kg particularly preferred. It will be evident to those skilled in the art that the number and frequency of administration will be dependent upon the response of the host. "Pharmaceutically acceptable carriers" for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid may be added as preservatives. *Id.* at 1449. In addition, antioxidants and suspending agents may be used. *Id.*

"Pharmaceutically acceptable salt" refers to salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid (acid addition salts) or an organic or inorganic base (base addition salts). The compounds of the present invention may be used in either the free base or salt forms, with both forms being considered as being within the scope of the present invention.

The pharmaceutical compositions that contain one or more nucleic acid constructs of the invention, for example, binding domain-immunoglobulin fusion protein encoding constructs (or their expressed products) may be in any form that allows for the composition to be administered to a patient. For example, the composition may be in the form of a solid, liquid or gas (aerosol). Typical routes of administration include, without limitation, oral, topical, parenteral (*e.g.*, sublingually or buccally), sublingual, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal, intracavernous, intrathecal, intrameatal, intraurethral injection or infusion techniques. The pharmaceutical composition is formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of one or more compounds of the invention in aerosol form may hold a plurality of dosage units.

For oral administration, an excipient and/or binder may be present. Examples are sucrose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose and ethyl cellulose. Coloring and/or flavoring agents may be present. A coating shell may be employed.

The composition may be in the form of a liquid, *e.g.*, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred compositions contain, in addition to one or more binding domain-immunoglobulin fusion construct or expressed product, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent, for example, may be included.

A liquid pharmaceutical composition as used herein, whether in the form of a solution, suspension or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such

as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

It may also be desirable to include other components in the preparation, such as delivery vehicles including but not limited to aluminum salts, water-in-oil emulsions, biodegradable oil vehicles, oil-in-water emulsions, biodegradable microcapsules, and liposomes. Examples of immunostimulatory substances (adjuvants) for use in such vehicles include N-acetylmuramyl-L-alanine-D-isoglutamine (MDP), lipopolysaccharides (LPS), glucan, IL-12, GM-CSF, gamma interferon and IL-15.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration and whether a sustained release is desired. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109. In this regard, it is preferable that the microsphere be larger than approximately 25 microns.

Pharmaceutical compositions may also contain diluents such as buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (*e.g.*, sucrose) as diluents.

As described above, the subject invention includes compositions capable of delivering nucleic acid molecules encoding binding domain-immunoglobulin fusion

proteins. Such compositions include recombinant viral vectors (*e.g.*, retroviruses (*see* WO 90/07936, WO 91/02805, WO 93/25234, WO 93/25698, and WO 94/03622), adenovirus (*see* Berkner, 1988 *Biotechniques* 6:616-627; Li *et al.*, 1993 *Hum. Gene Ther.* 4:403-409; Vincent *et al.*, *Nat. Genet.* 5:130-134; and Kolls *et al.*, 1994 *Proc. Natl. Acad. Sci. USA* 91:215-219), pox virus (*see* U.S. Patent No. 4,769,330; U.S. Patent No. 5,017,487; and WO 89/01973)), recombinant expression construct nucleic acid molecules complexed to a polycationic molecule (*see* WO 93/03709), and nucleic acids associated with liposomes (*see* Wang *et al.*, 1987 *Proc. Natl. Acad. Sci. USA* 84:7851). In certain embodiments, the DNA may be linked to killed or inactivated adenovirus (*see* Curiel *et al.*, 1992 *Hum. Gene Ther.* 3:147-154; Cotton *et al.*, 1992 *Proc. Natl. Acad. Sci. USA* 89:6094). Other suitable compositions include DNA-ligand (*see* Wu *et al.*, 1989 *J. Biol. Chem.* 264:16985-16987) and lipid-DNA combinations (*see* Felgner *et al.*, 1989 *Proc. Natl. Acad. Sci. USA* 84:7413-7417).

In addition to direct *in vivo* procedures, *ex vivo* procedures may be used in which cells are removed from a host, modified, and placed into the same or another host animal. It will be evident that one can utilize any of the compositions noted above for introduction of constructs of the invention, for example, binding domain-immunoglobulin fusion proteins or of binding domain-immunoglobulin fusion protein encoding nucleic acid molecules into tissue cells in an *ex vivo* context. Protocols for viral, physical and chemical methods of uptake are well known in the art.

Accordingly, the present invention is useful for treating a patient having a B cell disorder or a malignant condition, or for treating a cell culture derived from such a patient. As used herein, the term "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with cancer or a malignant condition, such as B cell lymphoma, or may be normal (*i.e.*, free of detectable disease and infection). A "cell culture" includes any preparation amenable to *ex vivo* treatment, for example a preparation containing immunocompetent cells or isolated cells of the immune system (including, but not limited to, T cells, macrophages, monocytes, B cells and dendritic cells). Such cells may be isolated by any of a variety of techniques well known to those of ordinary skill in the art (*e.g.*, Ficoll-hypaque density centrifugation). The cells may (but need not) have been isolated from a patient afflicted with a B cell disorder or a malignant condition, and may be reintroduced into a patient after treatment.

A liquid composition intended for either parenteral or oral administration should contain an amount of a construct of the invention, for example, a binding domain-immunoglobulin fusion protein encoding construct or expressed product, such that a suitable dosage will be obtained. Typically, this amount is at least 0.01 wt% of a binding domain-immunoglobulin fusion construct or expressed product in the composition. When intended for oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition. Preferred oral compositions contain between about 4% and about 50% of binding domain-immunoglobulin fusion construct or expressed product(s). Preferred compositions and preparations are prepared so that, for example, a parenteral dosage unit contains between 0.01 to 1% by weight of active compound.

The pharmaceutical composition may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment, or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, beeswax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. Topical formulations may contain a concentration of a construct of the invention, for example, a binding domain-immunoglobulin fusion construct or expressed product, of from about 0.1 to about 10% w/v (weight per unit volume).

The composition may be intended for rectal administration, in the form, *e.g.*, of a suppository that will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

In the methods of the invention, a construct of the invention, for example, a binding domain-immunoglobulin fusion encoding constructs or expressed product(s), may be administered through use of insert(s), bead(s), timed-release formulation(s), patch(es) or fast-release formulation(s).

Constructs of the invention, for example, antigen-binding constructs of the invention, may be administered or co-administered to an animal or patient in combination with, or at the same or about the same time, as other compounds. In one aspect, one or more constructs, including for example one or more antigen-binding constructs, are

administered to an animal or patient in conjunction with one or more chemotherapeutic compounds such as alkylating agents, nucleoside analogues, and the like. The administration or co-administration of one or more constructs, including one or more antigen-binding constructs, of the invention and one or more chemotherapeutic agents can be used for the treatment of tumors or cancer in an animal or patient. Exemplary cancers include, but are not limited to, head and neck cancer, breast cancer, colorectal cancer, gastric cancer, hepatic cancer, bladder cancer, cervical cancer, endometrial cancer, lung cancer (non-small cell), ovarian cancer, pancreatic cancer, prostate cancer; choriocarcinoma (lung cancer); hairy cell leukemia, chronic lymphocytic leukemia, acute lymphocytic leukemia (breast & bladder), acute myelogenous leukemia, meningeal leukemia, chronic myelogenous leukemia, erythroleukemia. More commonly the cancers treated include non-Hodgkin's lymphoma (osteogenic sarcoma, adult soft tissue sarcoma), T-cell lymphoma, chronic lymphocytic leukaemia, slowly growing non-Hodgkin's lymphomas, Hodgkin's lymphoma and ovarian cancer.

Examples of an alkylating agents that can be co-administered with one or more constructs, including one or more antigen-binding constructs, of the invention include mechlorethamine, chlorambucil, ifosfamide, melphalan, busulfan, carmustine, lomustine, procarbazine, dacardazine, cisplatin, carboplatin, mitomycin C, cyclophosphamide, isosfamide, , hexamethylmelamine, thiotepa, , and dacarbazine, and analogues thereof. See for example U.S. Pat. No. 3,046,301 describing the synthesis of chlorambucil, U.S. Pat. No. 3,732,340 describing the synthesis of ifosfamide, U.S. Pat. No. 3,018,302 for the synthesis of cyclophosphamide, U.S. Pat. No. 3,032,584 describing the synthesis of melphalan, and Braunwald et al., "Harrison's Principles of Internal Medicine," 15th Ed., McGraw-Hill, New York, NY, pp.536-544 (2001) for clinical aspects of cyclophosphamide, chlorambucil, melphalan, ifosfamide, procarbazine, hexamethylmelamine, cisplatin, and carboplatin. Examples of nucleoside analogues, include, but are not limited to, fludarabine pentostatin, methotrexate, fluorouracil, fluorodeoxyuridine, CB3717, azacitidine, cytarabine, floxuridine, mercaptopurine, 6-thioguanine, cladribine, and analogues thereof. One example is the combination of constructs, including antigen-binding constructs that bind CD20. This construct acts as a chemosensitising agent and works together with chemotherapeutic agents, such that less chemotherapeutic agents are necessary to achieve anti-tumor or anti-cancer effects. For

example, U.S. Pat. No. 3,923,785 describing the synthesis of pentostatin, U.S. Pat. No. 4,080,325 describing the synthesis of methotrexate, U.S. Pat. No. 2,802,005 describing the synthesis of fluorouracil, and Braunwald et al., "Harrison's Principles of Internal Medicine," 15th Ed., McGraw-Hill, New York, NY, pp.536-544 (2001) for clinical aspects
5 of methotrexate, 5-fluorouracil, cytosine arabinoside, 6-mercaptopurine, 6-thioguanine, and fludarabine phosphate.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered compounds that inhibit topoisomerase II or compounds that otherwise interact with nucleic acids in
10 cells. Such compounds include, for example, doxorubicin, epirubicin, etoposide, teniposide, mitoxantrone, and analogues thereof. In one example, this combination is used in treatment to reduce tumor cell contamination of peripheral blood progenitor cells (PBSC) in conjunction with high-dose chemotherapy and autologous stem cell support (HDC-ASCT). See U.S. Patent 6,586,428 to Geroni *et al.*

15 In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with therapeutic drugs. For example, Virulizin (Lorus Therapeutics), which is believed to stimulate the release of tumour necrosis factor, TNF-alpha, by tumour cells in vitro and stimulate activation of macrophage cells. This can be used in combination with one or more
20 constructs, including one or more antigen-binding constructs, of the invention to increase cancer cell apoptosis and treat various types of cancers including Pancreatic Cancer, Malignant Melanoma, Kaposi's Sarcoma (KS), Lung Cancer, Breast Cancer, Uterine, Ovarian and Cervical Cancer. Another example is CpG 7909 (Coley Pharmaceutical Group), which is believed to activate NK cells and monocytes and enhance ADCC. This
25 drug can be used in combination with cancer or tumor specific constructs, including antigen-binding constructs, of the invention, such as an anti-CD20 construct, to treat non-Hodgkin's lymphoma and other cancers.

One or more constructs, including one or more antigen-binding constructs, of the invention can also be combined with angiogenesis inhibitors to increase anti-tumor
30 effects. Angiogenesis is the growth of new blood vessels. This process allows tumors to grow and metastasize. Inhibiting angiogenesis can help prevent metastasis, and stop the spread of tumor cells. Angiogenesis inhibitors include, but are not limited to, angiostatin,

endostatin, thrombospondin, platelet factor 4, Cartilage-derived inhibitor (CDI), retinoids, Interleukin-12, tissue inhibitor of metalloproteinase 1, 2 and 3 (TIMP-1, TIMP-2, and TIMP-3) and proteins that block the angiogenesis signaling cascade, such as anti-VEGF (Vascular Endothelial Growth Factor) and IFN-alpha. Angiogenesis inhibitors can be administered or co-administered with tumor specific constructs, including antigen-binding constructs capable of mediating, for example, ADCC and/or complement fixation or chemotherapy-conjugated antigen-binding of the invention to combat various types of cancers, for example, solid tumor cancers such as lung and breast cancer.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with disease modifying anti-rheumatic agents (DMAR agents) for the treatment of rheumatoid arthritis, psoriasis, ulcerative colitis, systemic lupus erythematosus (SLE), Crohn's disease, ankylosing spondylitis, and various inflammatory disease processes. In such treatment, the constructs, for example, antigen-binding constructs, of the invention are commonly administered in conjunction with compounds such as azathioprine, cyclosporin, gold, hydroxychloroquine, methotrexate, penicillamine, sulphasalazine, and the like.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with agents or compounds that counteract the biological effects of interleukin-1, including for example interleukin-1 inhibitors and interleukin-1 receptor antagonist. It is thought that interleukin-1 has a role in the generation of rheumatoid arthritis (RA), inflammation, and the destruction of joints. IL-1 inhibitors can also be used in conjunction with the constructs, including antigen-binding constructs, of the invention to treat arthritis, inflammatory bowel disease, sepsis and septic shock, ischemic injury, reperfusion, ischemic brain injury such as cerebral palsy and multiple sclerosis. See U.S. Patent No. 6,159,460 to Thompson *et al.* In another aspect, for example, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered to an animal or patient in conjunction with one or more glucocorticoids for example, methylprednisilone, dexamethasone, hydrocortisone, and the like. Glucocorticoids have been used to induce apoptosis and inhibit growth, independent of ADCC and CDC. These compounds can be combined with constructs, including antigen-binding constructs, of the invention capable of inducing apoptosis in cancer cells. In one example is the anti-CD20, and anti-CD40

antigen-binding constructs, which can be used to induce apoptosis in B-cells, are combined with glucocorticoids to treat B-cell non-Hodgkin's lymphoma (NHL).

5 In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with p38 inhibitors or antagonists. The p38 mitogen-activated protein kinase pathway is involved in a number of cellular processes instrumental to the development of rheumatoid arthritis. For example, the activation and infiltration of leukocytes as well as the production of inflammatory cytokines are p38-dependent processes.

10 In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention are administered or co-administered with compounds that promote the differentiation and proliferation of B-cells. Cytokines such as \ interleukin-4 (IL-4) and interleukin-6 (IL-6), in addition to other biological activities, have been shown to stimulate antibody synthesis and secretion by activated B lymphocytes. In a particular aspect of the invention, constructs, including antigen-binding constructs that
15 recognize and bind CD20 are co-administered with one or more of interleukin-4 (IL-4) and interleukin-6 (IL-6).

In another aspect one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with
Interleukin-2 (IL-2). Interleukin 2 (IL-2) is a lymphokine that increases production of
20 effector cells, such as CD4+ T-helper cells, CD8 cytotoxic cells, antibody producing B cells, natural killer cells (NK), and monocytes/macrophages. IL-2 helps produce T-cells, which in turn secrete more of the IL-2 (an "autocrine loop"). IL-2 can be used to augment antibody-dependent cell-mediated cytotoxicity (ADCC) and immunotherapies associated with constructs of the invention. In one example, an anti-CD20 construct of the invention
25 and IL-2 are used to treat patients with relapsed or refractory follicular non-Hodgkin's lymphoma. In another example IL-2 is administered or co-administered with HIV immunotherapies to help with T cell recovery.

In another aspect one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with
30 Interleukin-12 (IL-12). IL-12 is know to enhance cytolytic T-cell responses, promote the development of helper T cells, enhance the activity of natural killer (NK) cells, and induces the secretion of IFN- γ in T and NK cells. IL-12 also increases many helper and effector

cells that mediate apoptosis. In another aspect of the invention, one or more constructs, including one or more antigen-binding constructs, are administered or co-administered with IL-12 in the treatment of an animal or patient with a tumor or cancer. For example, a construct, including an antigen-binding construct, of the invention that binds CD20 combined with IL-2 for the treatment of a patient with B-cell non-Hodgkin's lymphoma (NHL).

One or more constructs, including one or more antigen-binding constructs, of the invention can also be combined with immunomodulators to boost the efficacy of the antigen-binding constructs of the invention. Immunomodulators include, but are not limited to, Colony Stimulating Factors (CSF), Tumor necrosis Factors (TNF), and Interferons (IFN).

CSFs can include granulocyte-macrophage CSF (GM-CSF), granulocyte-CSF (G-CSF), and macrophage CSF (M-CSF). GM-CSF is thought to regulate the development of neutrophils, macrophages, monocytes and eosinophils. G-CSF has been shown to induce neutrophil production, and M-CSF production. M-CSF has been shown to stimulate macrophages and monocytes. The use of CSFs to treat neutropenia in cancer patients has been long established. In one example, constructs, including antigen-binding constructs, of the invention can be combined with GM-CSF, G-CSF or combinations thereof in order to accelerate recovery from neutropenia in patients after bone marrow trans-plantation and chemotherapy. Neutrophils play a major role in fighting microbes such as bacterial, fungi and parasites. Patients with neutropenia are particularly susceptible to bacterial and wide spread fungal infections. In another example, a construct, including an antigen binding construct, of the invention can be combined with GM-CSF-treated neutrophils, monocytes and macrophages to increase activity against bacteria, fungi, etc, including the dreaded *Pneumocystis carinii*.

An example of an IFN is interferon alpha (IFN- α). IFN- α is made naturally by some types of white blood cell as part of the immune response when the body reacts to cancers or viral infections. It has two main modes of attack, interfering with growth and proliferation of cancer cells and it boosting the production of killer T cells and other cells that attack cancer cells. Interferon is also thought to facilitate cancer cells to put out chemical signals that make them better targets for the immune system, and has been used in recent years for several different types of cancer, particularly kidney cancer, melanoma,

multiple myeloma, and some types of leukemia. It is also used to treat viral infections such as hepatitis. Interferon-alpha2a, for example, enhances ADCC and can be combined with one or more constructs, including antigen-binding constructs, of the invention to increase the efficiency of ADCC activity associated with the construct. In another example, one or more constructs, including one or more antigen-binding constructs of the invention are administered or co-administered to an animal or patient with interferon-gamma (IFN- γ), which has been show to increase the number of anti-CD20 antigens on B cells and bone marrow plasma cells (BMPC). This is particularly useful for the treatment of patients with multiple myelomas, which have a reduced expression of CD20 in their B cells and bone marrow plasma cells (BMPC). Accordingly, the treatment of multiple myeloma patients with constructs, including antigen-binding constructs of the invention, in particular constructs that bind CD20, may be usefully co-administered in conjunction with IFN- γ

TNF is a class of natural chemicals with anticancer properties. One example of a TNF is TNF- alpha. TNF-alpha has also been shown to have synergistic effects with IFN-gamma and IL-12. In another example, TNF can be administered or co-administered with one or more tumor specific constructs, including one or more antigen-binding constructs, of the invention, and include chemotherapy-conjugated antigen binding constructs of the invention, together with IFN-gamma, IL-12 or various combinations thereof. TNF is also known to be an inflammatory regulation molecule. TNF-alpha antibodies or antagonist(s) can be combined with anti-T cell constructs, including antigen-binding constructs, of the invention to treat patients with rheumatoid arthritis, psoriasis, ulcerative colitus, systemic lupus erythematosus (SLE), Crohn's disease, ankylosing spondylitis, and various inflammatory disease processes.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with another antibody or antigen-binding construct of the invention. One example is a construct, for example, an antigen-binding construct of the invention capable of binding CD20 combined with a construct capable of binding CD22, CD19 or combinations thereof. This combination is effective as a treatment for indolent and aggressive forms of B-cell lymphomas, and acute and chronic forms of lymphatic leukemias. See U.S. Patent 6,306,393 to Goldberg. In another example, constructs, including antigen-binding constructs, of the invention are co-administered with other constructs such as antigen-

binding constructs of the invention that aid in mediating apoptosis. For example, a combination of one or more constructs, including one or more antigen-binding constructs of the invention capable of binding CD28, CD3, CD20 or a combination thereof. The combination of anti-CD28 and CD3 provides a method for prolonged proliferation of T-cells. See U.S. Patent No. 6,352,694 to June et al.. This prolonged T-cell proliferation increases the efficiency immune dependent cytotoxicity, particularly those associated with anti-CD20.

In another aspect, constructs, including antigen-binding constructs, of the invention can be administered or co-administered with one or more T-cell regulatory molecules. One example is a combination with interleukin-12 (IL-12). The IL-12 cytokine stimulates cell-mediated immunity, has angiostatic activity, and possesses significant anti-tumor effects in a variety of tumor models. IL-12 has also been shown to stimulate the production of interferon-gamma (IFN- γ). Accordingly, the treatment of multiple myeloma patients with one or more constructs, including one or more antigen-binding constructs, of the invention, in particular those that bind CD20, is expected to be more efficacious when co-administered in conjunction with IL-12. In another example, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with a binding-domain construct of the invention other protein capable of binding CTLA-4 to enhance the anti-tumor immune response, by inhibiting the downregulation of T-cell activation.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be combined with gene therapies. In one example, a chemotherapy-conjugated construct of the invention is administered or co-administered with the Bcl-2 antisense oligonucleotide. Bcl-2 is associated with tumor resistance to anti-cancer therapies, and its believed to blocking chemotherapy-induced cell death. In another example one or more constructs, including one or more antigen-binding constructs, of the invention is administered or co-administered with an adenovirus for delivery of a "suicide gene." The adenovirus inserts the gene directly into the tumor cells, which makes these cells sensitive to an otherwise ineffective drug. Drug treatment then destroys the tumor cells, while leaving healthy cells untouched. However, once therapy is complete stray cancer cells that escaped therapy can reestablish and metastasize. Combining gene therapy

with one or more constructs, including one or more antigen-binding constructs, will help kill stray cancer cells and minimize cancer reoccurrence.

5 A similar combination can be used with palliative (non-radical) operations to surgically remove tumors. In this example one or more constructs, including one or more antigen-binding constructs, of the invention can be administered before and after surgical extractions of tumors in order to increase the immune response and reduce the likelihood of reoccurrence by killing any cancer cells that were not removed during the surgery.

10 Another aspect combines a cancer or antigen vaccine and T-cell regulator molecules. For example, the binding portion, for example, an antigen-binding portion, of a construct can be specific for a cancer cell or antigen, or a protein fragment from a cancer cell or antigen. This can help mediate an immune response against a particular tumor or antigen. Such constructs can be combined with T-cell regulators to increase the efficiency of the immune response.

15 In another example, one or more constructs, including one or more antigen-binding constructs, of the invention is administered or co-administered with retinoids. Retinoids include Vitamin A and its derivatives, which have the ability to stop cells from dividing and cause them to differentiate. Vitamin A is combined with an anti-cancer construct(s), including antigen-binding construct(s), of the invention to combat various forms of cancer.

20 The terms "binding construct" and "antigen-binding construct" as used herein may refer to, for example, engineered polypeptides, recombinant polypeptides, synthetic, semi-synthetic or other fusion proteins that are capable of binding a target, for example, an antigen. Antigen-binding constructs of the invention may be used in various applications, including those within the variety of uses to which antibodies or related immunoglobulin-type constructs may be put. Constructs, including antigen-binding constructs of the invention can be used in *in vivo* and *in vitro* experiments for therapeutic, diagnostic, research, and other purposes. Such uses include, for example, the following.

25 Constructs, including antigen-binding constructs of the invention may be used for immunohistochemistry applications. For example, they may be used for immunolocalization of a particular antigen or group of antigens in a tissue. Tissue can be fixed and incubated with antigen-binding constructs of interest. These constructs can then

be localized using a secondary antibody or binding construct of the invention coupled to a label, for example, to a gold particle or an enzyme that gives a chemical reaction, like horseradish peroxidase or beta-galactosidase. A secondary antibody or binding construct is frequently made that is reactive against, for example, a portion of the primary binding
5 construct. Thus, for example, if the primary binding construct has a human tail portion, the secondary antibody or binding construct could be, for example, a rabbit anti-mouse antibody or antigen-binding construct that has been linked to beta-galactosidase. Alternatively the antibody or binding construct of the invention can be purified and then conjugated to another molecule to produce a fluorescent antibody or binding construct.

10 Constructs, including antigen-binding constructs of the invention can also be used to detect the location of an antigen or antigens on the surface of cells or to detect the location of intracellular materials using, for example, Immunoelectron Microscopy. Electron dense materials such as ferritin or colloidal gold, for example, can be conjugated to an antigen-binding construct. Scanning electron microscopy can be used to detect the
15 localization of the antigen/binding construct complex.

Constructs, including antigen-binding constructs of the invention may also be used to quantitate the presence of an antigen or antigens using one of a variety of immunoassay formats, for example, a radioimmunoassay (RIA) format or an enzyme-linked immunosorbent assay (ELISA) format. There are many variants of these
20 approaches, but those are based on a similar idea. For example, if an antigen can be bound to a solid support or surface, or is in solution, it can be detected by reacting it with a specific antigen-binding construct of the invention. The presence or amount of the construct can then be detected or quantitated by reacting it with, for example, either a secondary antibody or a second antigen-binding construct of the invention by incorporating
25 a label directly into the primary antibody. Alternatively, for example, an antigen-binding polypeptide of the invention can be bound to a solid surface and the antigen added. A second antibody or antigen-binding polypeptide(s) of the invention that recognizes a distinct epitope on the antigen can then be added and detected. This technique is commonly referred to as a "sandwich assay", which is frequently used to avoid problems
30 of high background or non-specific reactions, among other reasons.

Because the binding constructs of the invention can have high affinity/affinities and/or selectivity/selectivities for a particular epitope or epitopes, they

can also be used as affinity reagents, for example, in protein or antigen purification. In one example of such a process, antigen-binding constructs of the invention are immobilized on a suitable support, for example, Sephadex resin or filter paper. The immobilized construct is exposed to a sample containing, or suspected of containing, a target protein(s) or antigen(s). The support is rinsed with a suitable buffer that will remove unwanted materials. The support is washed with another buffer that will release the bound protein(s) or antigen(s).

Because particular binding constructs of the invention can bind to proteins or other antigens with high affinity and selectivity they can also be used as a criterion for the importance of a particular enzyme or other macromolecule in a particular reaction. If an antigen-binding construct of the invention can interfere with a reaction in a solution, this will indicate that the construct may be binding specifically to a protein or other antigenic material involved in that reaction.

Constructs, including antigen-binding constructs of the invention can also be used as receptor blockers or inhibitors or antagonists.

Constructs, including antigen-binding constructs of the invention can also be used in identifying and studying the function(s) of proteins. If an antigen-binding construct of the invention reacts with a specific protein, for example, that protein can subsequently be precipitated from solution, for example. Precipitation is typically performed by using a secondary antibody or antigen-binding construct of the invention that links primary complexes together. Alternatively, the complex can be removed by reacting the solution with either protein A or, for example, depending on the construct, an anti-Fc antibody, for example, which has been attached to beads, for example, so that can be easily removed from the solution.

Constructs, including antigen-binding constructs of the invention can also be used in conjunction with gel-shift experiments to identify specific nucleic acid-binding proteins such as DNA-binding proteins. For example, DNA-binding proteins can be assayed by their ability to bind with high affinity to a particular oligonucleotide. The mobility of an oligonucleotide associated with the protein is far different than the mobility of a free oligonucleotide and results in a gel migration pattern and signal that is commonly referred to as a gel shift. The addition of the construct to the binding assay can have either of two effects. If the construct binds to a region of a protein not involved in DNA binding

it can result in a complex that has even a slower mobility and is detected as a greater shift in mobility (a super-shift). Alternatively, if the construct binds to a region of the protein involved in recognizing the DNA then it can disrupt the binding and eliminate the shift. In either case, the data from these experiments can serve as a criterion to identify a DNA-binding protein, for example.

It is also possible to use constructs, including antigen-binding constructs of the invention to detect a protein by western blotting after fractionation by SDS-PAGE, for example. Once fractionated proteins are transferred to a membrane such as a nitrocellulose sheet, they are exposed to a particular antigen-binding construct of the invention that specifically recognizes, or recognizes to a desired degree of selectivity, proteins immobilized to the blot. This allows particular proteins to be identified. This approach is particularly useful if the mobility of the protein changes during an experiment. For example, incorporation of a phosphate or a carbohydrate, or cleavage of the protein, results in a change in mobility that can be followed in straightforward manner by western analysis. With appropriate controls, this approach can be used to measure the abundance of a protein in response to experimental manipulations.

The combination of SDS gels and immunoprecipitation can also be extremely effective. If a particular protein can be immunoprecipitated in a solution, both supernatant and precipitated fractions can be separated on an SDS gel and studied using an antigen-binding construct(s) of the invention.

Sometimes a binding construct of the invention directed against one protein will also precipitate a second protein that interacts with the first protein. The second protein, as well as the first, can then be seen by staining the gel or by autoradiography. This relationship is frequently the first indication that a protein functions as part of a complex and it can also be used to demonstrate a physical interaction of two proteins that are hypothesized to interact on the basis of other evidence (*e.g.*, a two hybrid screen or a suppressor mutation). This approach can be combined with western blotting analysis in several extremely effective ways.

Thus, for example, antigen-binding constructs of the invention can be used in a combination of immunoprecipitation and western analysis in the study, for example, of signal transduction and protein processing. For example, an immunoprecipitated protein can be subsequently studied by western analysis using a different antibody or antigen-

binding construct of the invention that binds to the protein. The most useful of are those that are directed against particular structural determinants that may be present in a protein. Thus, an antibody or antigen-binding construct of the invention directed against a region of the protein that undergoes proteolytic processing can be useful to follow proteolytic processing. Additionally, a construct of the invention or a mixture of antigen-binding constructs of the invention that recognize phosphorylated peptides (*e.g.*, anti PY (phosphorylated tyrosine) can be used to follow the extent of phosphorylation of a protein (using western analysis) after it is precipitated, or *visa versa*. Glycosylation reactions can also be followed by antigen-binding constructs of the invention directed against a carbohydrate epitope (or by lectins, *i.e.*, proteins that recognize carbohydrates). Likewise, some antigen-binding constructs of the invention can be made that specifically recognize a phosphorylated epitope, for example, that will recognize a tyrosine or a serine residue after phosphorylation, but will not bind (or detectably bind) the epitope in the absence of phosphate. This approach can be used to determine the phosphorylation state of a particular protein. For example, the phosphorylation of CREB (the cAMP response element binding protein) can be followed by an antibody that specifically recognizes an epitope in a way that is dependent on the phosphorylation of serine 133.

Constructs, including antigen-binding constructs of the invention can also be used to screen expression libraries to isolate candidate polynucleotides that express or present a particular epitope, or that have a particular affinity or expression characteristic.

Constructs, including antigen-binding constructs of the invention that bind to a cell surface can also be used as a marker to quantitate the fraction of cells expressing that marker using flow cytometry. If different antigen binding constructs of the invention / fluorescent dye combinations are used, for example, the fraction of cells expressing several antigens can be determined.

Constructs, including antigen-binding constructs of the invention that function like anti-idiotypic antibodies, *i.e.*, antibodies against the binding domain of another antibody, can be used in any of a number of methods in which it would be desirable or useful to mimic the structure of an antigen. Such uses include, for example, uses as cancer vaccines (including antigen-binding constructs of the invention that incorporate a molecular adjuvant), as probes for receptors, as receptor agonists, as receptor antagonists, as receptor blockers or inhibitors, and so on.

In another aspect, constructs, including antigen-binding constructs of the invention may bispecific and thus capable of binding to two distinct epitopes, which may be present on the same or different cell types.

In vivo uses of constructs of the invention, including antigen-binding constructs, include therapy, alone or in combination with one or more other therapies, for various diseases including cancers as well as B-cell disorders including autoimmune diseases. In some cases the constructs of the invention are administered to a patient. In other cases, the construct may be coupled to another molecule by techniques known in the art, for example, a fluorescent molecule to aid in imaging a target, or a therapeutic drug and/or a toxin to aid in killing a target.

For example, a labeling molecule or atom can be conjugated or otherwise linked to the antigen-binding construct of the invention to aid in imaging or as a diagnostic agent. These include, but are not limited to enzymatic labels, radioisotopes or radioactive compounds or elements, fluorescent compounds or metals, chemiluminescent compounds and bioluminescent compounds. Thus, binding constructs or antigen-binding constructs of the invention can be conjugated to a drug, which allows specific drug targeting and increased efficiency once the drug reaches the target. This facilitates drug therapy while reducing systemic toxicity and side effects. This allows use of drugs that would otherwise be unacceptable when administered systemically. Dosage will depend on the potency of the drug and the efficiency of the carrier construct. Other examples of *in vivo* uses include the use of binding constructs or antigen-binding constructs of the invention in which a toxin is chemically linked or conjugated to an polypeptide of the invention to form, for example, molecules that may be termed "immunoconjugates" or "immunotoxins." Typically, for example, such a toxin may include one or more radioisotopes (for example, Iodine-131, Yttrium-90, Rhenium-186, Copper-67, and/or Bismuth-212), natural toxins, chemotherapy agents, biological response modifiers, or any other substance that is capable of assisting in damaging or killing a target cell, inhibiting target cell replication, or is effective in disrupting a desired cellular function in a target cell.

The toxin portion of the immunotoxin can be derived from various sources. Toxins are commonly derived from plants or bacteria, but toxins of human origin or synthetic toxins can be used as well, for example. Examples of toxins derived from bacteria or plants include, but are not limited to, abrin, α -sarcin, diphtheria toxin, ricin,

5 saporin, and *pseudomonas* exotoxin. Examples of mammalian enzymes include, but are not limited to, ribonucleases (RNase) and deoxyribonucleases. Numerous immunotoxins that may be used with one or more constructs of the invention have been described in the art. See, for example, U.S. Pat. No. 4,753,894 to Frankel *et al.*; U.S. Pat. No. 6,099,842 to Pastan *et al.*; Nevelle, *et al.*, 1982 *Immunol Rev.* 62:75-91; Pastan *et al.*, 1992 *Ann Rev Biochem* 61:331-354; Chaudary *et al.*, 1989 *Nature* 339:394; and Batra *et al.*, 1991 *Mol. Cell. Biol.* 11:2200. Modified toxins described herein and those described in the various publications are also within the scope of the instant invention.

10 Generally, the immunotoxins and other therapeutic agents of this invention are administered at a concentration that is therapeutically effective to treat or prevent a particular disease, disorder, or condition, such as for the treatment of tumors and malignancies, the treatment of autoimmune diseases, allergies and inflammation, *etc.* This effective dosage and mode of administration will depend on the animal or patient being treated, the disease or condition being treated, the strength of the immunoconjugates or
15 immunotoxins and the efficiency of the conjugate. To accomplish this goal, the immunotoxins may be formulated using a variety of acceptable formulations and excipients known in the art. Typically, for example, the immunotoxins are administered by injection, either intravenously or intraperitoneally. Methods to accomplish this administration are known to those of ordinary skill in the art. In another aspect, the invention includes
20 topically or orally administered compositions such as an aerosol or cream or patch that may be capable of transmission across mucous membranes.

Formulants may be added to an immunoconjugates or immunotoxins of the invention before administration to a patients being treated. A liquid formulation is most common, but other formulations are within the scope of the invention. The formulants may
25 include for example oils, polymers, vitamins, carbohydrates, amino acids, salts, buffers, albumin, surfactants, or bulking agents. Carbohydrates can include sugar or sugar alcohols such as mono, di, or polysaccharides, or water-soluble glucans. The saccharides or glucans can include for example fructose, dextrose, lactose, glucose, mannose, sorbose, xylose, maltose, sucrose, dextran, pullulan, dextrin, alpha and beta cyclodextrin, soluble starch,
30 hydroxethyl starch and carboxymethylcellulose, or mixtures thereof. "Sugar alcohol" may be defined as a C₄ to C₈ hydrocarbon having an -OH group and includes, for example, galactitol, inositol, mannitol, xylitol, sorbitol, glycerol, and arabitol. These sugars or sugar

alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used as long as the sugar or sugar alcohol is soluble in the aqueous preparation. In one aspect, the sugar or sugar alcohol concentration is between 0.5 w/v % and 15 w/v %, typically between 1.0 w/v % and 7.0 w/v %, more typically between 2.0 and 5 6.0 w/v %.

Exemplary amino acids include levorotary (L) forms of camitine, arginine, and betaine; however, other amino acids may be added. Commonly used polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, for example, or polyethylene glycol (PEG) with an average molecular weight between 10 3,000 and 5,000, for example. A buffer can be used in the composition to minimize pH changes in the solution before lyophilization or after reconstitution. Any physiological buffer may be used, but citrate, phosphate, succinate, and glutamate buffers or mixtures thereof are more commonly utilized. The concentration can be, for example, from 0.01 to 0.3 molar. Higher or lower concentrations may be used.

15 Immunotoxins of the invention can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Exemplary polymers and methods to attach them to peptides are referenced in U.S. Pat. Nos. 4,766,106 to Katre *et al.*; 4,179,337 to Davis *et al.*; 4,495,285 to Shimizu *et al.*; and 4,609,546 to Hiratani.

20 The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

CLONING OF THE 2H7 VARIABLE REGIONS AND CONSTRUCTION

AND SEQUENCING OF 2H7scFv-IG

25 This Example illustrates the cloning of cDNA molecules that encode the heavy chain and light chain variable regions of the monoclonal antibody 2H7. This Example also demonstrates the construction, sequencing, and expression of 2H7scFv-Ig.

Prior to harvesting, cells expressing 2H7 monoclonal antibody that specifically bound to CD20 were kept in log phase growth for several days in RPMI 1640 media Invitrogen/Life Technologies, Gaithersburg, MD) supplemented with glutamine, pyruvate, DMEM non-essential amino acids, and penicillin-streptomycin. Cells were 30 pelleted by centrifugation from the culture medium, and 2×10^7 cells were used to prepare

RNA. RNA was isolated from the 2H7-producing hybridoma cells using the Pharmingen (San Diego, CA) total RNA isolation kit (Catalog # 45520K) according to the manufacturer's instructions accompanying the kit. One microgram (1 µg) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA and 300 ng
 5 random primers were combined and denatured at 72 °C for 10 minutes prior to addition of enzyme. Superscript II reverse transcriptase (Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 µl in the presence of 5X second strand buffer and 0.1 M DTT provided with the enzyme. The reverse transcription reaction was allowed to proceed at 42°C for one hour.

10 The 2H7 cDNA generated in the randomly primed reverse transcriptase reaction and V region specific primers were used to amplify by PCR the variable regions for the light and heavy chain of the 2H7 antibody. The V region specific primers were designed using the published sequence (Genbank accession numbers M17954 for V_L and M17953 for V_H) as a guide. The two variable chains were designed with compatible end
 15 sequences so that an scFv could be assembled by ligation of the two V regions after amplification and restriction enzyme digestion.

A (Gly₄Ser)₃ peptide linker to be inserted between the two V regions was incorporated by adding the extra nucleotides to the antisense primer for the V_L of 2H7. A Sac I restriction site was also introduced at the junction between the two V regions. The
 20 sense primer used to amplify the 2H7 V_L, that included a HindIII restriction site and the light chain leader peptide was 5'-gtc aag ctt gcc gcc atg gat ttt caa gtg cag att ttt cag c-3' (SEQ ID NO:___). The antisense primer was 5'-gtc gtc gag ctc cca cct cct cca gat cca cca ccg ccc gag cca ccg cca cct ttc agc tcc agc ttg gtc cc-3' (SEQ ID NO:___). The reading frame of the V region is indicated as a bold, underlined codon. The Hind III and SacI sites
 25 are indicated by underlined italicized sequences.

The V_H domain was amplified without a leader peptide, but included a 5' SacI restriction site for fusion to the V_L and a BclI restriction site at the 3' end for fusion to various tails, including the human IgG1 Fc domain and the truncated forms of CD40 ligand, CD154. The sense primer was 5'-gct gct gag ctc tca ggc tta tct aca gca agt ctg g-3'
 30 (SEQ ID NO:___). The SacI site is indicated in italicized and underlined font, and the reading frame of the codon for the first amino acid of the V_H domain is indicated in bold, underlined type. The antisense primer was 5'-gtt gtc tga tca gag acg gtg acc gtg gtc cc-3'

(SEQ ID NO:___). The BclI site is indicated in italicized, underlined type, and the last serine of the V_H domain sequence is indicated in bold, underlined type.

The scFv-Ig was assembled by inserting the 2H7 scFv HindIII-BclI fragment into pUC19 containing the human IgG1 hinge, CH2, and CH3 regions, which was digested with restriction enzymes, HindIII and BclI. After ligation, the ligation products were transformed into DH5 α bacteria. Positive clones were screened for the properly inserted fragments using the SacI site at the V_L-V_H junction of 2H7 as a diagnostic site. The 2H7scFv-Ig cDNA was subjected to cycle sequencing on a PE 9700 thermocycler using a 25-cycle program by denaturing at 96 °C for 10 seconds, annealing at 50 °C for 30 seconds, and extending at 72°C for 4 minutes. The sequencing primers were pUC forward and reverse primers and an internal primer that annealed to the CH2 domain human in the IgG constant region portion. Sequencing reactions were performed using the Big Dye Terminator Ready Sequencing Mix (PE-Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Samples were subsequently purified using Centriscap columns (Catalog # CS-901, Princeton Separations, Adelphia, N.J.), the eluates dried in a Savant vacuum dryer, denatured in Template Suppression Reagent (PE-ABI), and analyzed on an ABI 310 Genetic Analyzer (PE-Applied Biosystems). The sequence was edited, translated, and analyzed using Vector Nti version 6.0 (Informax, North Bethesda, MD). Figure 1 shows the cDNA and predicted amino acid sequence of the 2H7scFv-Ig construct.

EXAMPLE 2

EXPRESSION OF 2H7 SCFV-IG IN STABLE CHO CELL LINES

This Example illustrates expression of 2H7scFv-Ig in a eukaryotic cell line and characterization of the expressed 2H7scFv-Ig by SDS-PAGE and by functional assays, including ADCC and complement fixation.

The 2H7scFv-Ig HindIII-XbaI (~1.6 kb) fragment with correct sequence was inserted into the mammalian expression vector pD18, and DNA from positive clones was amplified using QIAGEN plasmid preparation kits (QIAGEN, Valencia, CA). The recombinant plasmid DNA (100 μ g) was then linearized in a nonessential region by digestion with AscI, purified by phenol extraction, and resuspended in tissue culture media, Excell 302 (Catalog # 14312-79P, JRH Biosciences, Lenexa, KS). Cells for transfection, CHO DG44 cells, were kept in logarithmic growth, and 10⁷ cells harvested for each

transfection reaction. Linearized DNA was added to the CHO cells in a total volume of 0.8 ml for electroporation.

Stable production of the 2H7 scFv-Ig fusion protein (SEQ. ID NO:10) was achieved by electroporation of a selectable, amplifiable plasmid, pD18, containing the 2H7 scFv-Ig cDNA under the control of the CMV promoter, into Chinese Hamster Ovary (CHO) cells (all cell lines from American Type Culture Collection, Manassas, VA, unless otherwise noted). The 2H7 expression cassette was subcloned downstream of the CMV promoter into the vector multiple cloning site as a ~1.6 kb HindIII-XbaI fragment. The pD18 vector is a modified version of pcDNA3 encoding the DHFR selectable marker with an attenuated promoter to increase selection pressure for the plasmid. Plasmid DNA was prepared using Qiagen maxiprep kits, and purified plasmid was linearized at a unique AscI site prior to phenol extraction and ethanol precipitation. Salmon sperm DNA (Sigma-Aldrich, St. Louis, MO) was added as carrier DNA, and 100 µg each of plasmid and carrier DNA was used to transfect 10^7 CHO DG44 cells by electroporation. Cells were grown to logarithmic phase in Excell 302 media (JRH Biosciences) containing glutamine (4mM), pyruvate, recombinant insulin, penicillin-streptomycin, and 2X DMEM nonessential amino acids (all from Life Technologies, Gaithersburg, Maryland), hereafter referred to as "Excell 302 complete" media. Media for untransfected cells also contained HT (diluted from a 100X solution of hypoxanthine and thymidine) (Invitrogen/Life Technologies). Media for transfections under selection contained varying levels of methotrexate (Sigma-Aldrich) as selective agent, ranging from 50 nM to 5 µM. Electroporations were performed at 275 volts, 950 µF. Transfected cells were allowed to recover overnight in non-selective media prior to selective plating in 96 well flat bottom plates (Costar) at varying serial dilutions ranging from 125 cells/well to 2000 cells/well. Culture media for cell cloning was Excell 302 complete, containing 100 nM methotrexate. Once clonal outgrowth was sufficient, serial dilutions of culture supernatants from master wells were screened for binding to CD20-CHO transfected cells. The clones with the highest production of the fusion protein were expanded into T25 and then T75 flasks to provide adequate numbers of cells for freezing and for scaling up production of the 2H7scFvIg. Production levels were further increased in cultures from three clones by progressive amplification in methotrexate containing culture media. At each successive passage of cells, the Excell 302 complete

media contained an increased concentration of methotrexate, such that only the cells that amplified the DHFR plasmid could survive.

Supernatants were collected from CHO cells expressing the 2H7scFv-Ig, filtered through 0.2 μ m PES express filters (Nalgene, Rochester, NY) and were passed
5 over a Protein A-agarose (IPA 300 crosslinked agarose) column (Repligen, Needham, MA). The column was washed with PBS, and then bound protein was eluted using 0.1 M citrate buffer, pH 3.0. Fractions were collected and eluted protein was neutralized using 1M Tris, pH 8.0, prior to dialysis overnight in PBS. Concentration of the purified 2H7scFv-Ig (SEQ ID NO:__) was determined by absorption at 280 nm. An extinction
10 coefficient of 1.77 was determined using the protein analysis tools in the Vector Nti Version 6.0 Software package (Informax, North Bethesda, MD). This program uses the amino acid composition data to calculate extinction coefficients.

Production levels of 2H7scFv-Ig by transfected, stable CHO cells were analyzed by flow cytometry. Purified 2H7scFv-Ig to CHO cells was allowed to bind to
15 CHO cells that expressed CD20 (CD20 CHO) and analyzed by flow cytometry using a fluorescein-conjugated anti-human IgG second step reagent (Catalog Numbers H10101 and H10501, CalTag, Burlingame, CA). Figure 2 (top) shows a standard curve generated by titration of 2H7scFv-Ig binding to CD20 CHO. At each concentration of 2H7scFv-Ig, the mean brightness of the fluorescein signal in linear units is shown. Supernatants collected
20 from T flasks containing stable CHO cell clones expressing 2H7scFv-Ig were then allowed to bind to CD20 CHO and the binding was analyzed by flow cytometry. The fluorescein signal generated by 2H7scFv-Ig contained in the supernatants was measured and the 2H7scFv-Ig concentration in the supernatants was calculated from the standard curve (Figure 2, bottom).

25 Purified 2H7scFv-Ig (SEQ ID NO:__) was analyzed by electrophoresis on SDS-Polyacrylamide gels. Samples of 2H7scFv-Ig, purified by independent Protein A Agarose column runs, were boiled in SDS sample buffer without reduction of disulfide bonds and applied to SDS 10% Tris-BIS gels (Catalog # NP0301, Novex, Carlsbad, CA). Twenty micrograms of each purified batch was loaded on the gels. The proteins were
30 visualized after electrophoresis by Coomassie Blue staining (Pierce Gel Code Blue Stain Reagent, Catalog #24590, Pierce, Rockford, IL), and destaining in distilled water. Molecular weight markers were included on the same gel (Kaleidoscope Prestained

Standards, Catalog # 161-0324, Bio-Rad, Hercules, CA). The results are presented in Figure 3. The numbers above the lanes designate independent purification batches. The molecular weights in kilodaltons of the size markers are indicated on the left side of the figure. Further experiments with alternative sample preparation conditions indicated that reduction of disulfide bonds by boiling the protein in SDS sample buffer containing DTT or 2-mercaptoethanol caused the 2H7scFv-Ig to aggregate.

Any number of other immunological parameters may be monitored using routine assays that are well known in the art. These may include, for example, antibody dependent cell-mediated cytotoxicity (ADCC) assays, secondary *in vitro* antibody responses, flow immunocytofluorimetric analysis of various peripheral blood or lymphoid mononuclear cell subpopulations using well established marker antigen systems, immunohistochemistry or other relevant assays. These and other assays may be found, for example, in Rose *et al.* (Eds.), *Manual of Clinical Laboratory Immunology*, 5th Ed., 1997 American Society of Microbiology, Washington, DC.

The ability of 2H7scFv-Ig to kill CD20 positive cells in the presence of complement was tested using B cell lines Ramos and Bjab. Rabbit complement (Pel-Freez, Rogers, AK) was used in the assay at a final dilution of 1/10. Purified 2H7scFv-Ig was incubated with B cells and complement for 45 minutes at 37 °C, followed by counting of live and dead cells by trypan blue exclusion. The results in Figure 4A show that in the presence of rabbit complement, 2H7scFv-Ig lysed B cells expressing CD20.

The ability of 2H7scFv-Ig to kill CD20 positive cells in the presence of peripheral blood mononuclear cells (PBMC) was tested by measuring the release of ⁵¹Cr from labeled Bjab cells in a 4-hour assay using a 100:1 ratio of PBMC to Bjab cells. The results shown in Figure 4B indicated that 2H7scFv-Ig can mediate antibody dependent cellular cytotoxicity (ADCC) because the release of ⁵¹Cr was higher in the presence of both PBMC and 2H7scFv-Ig than in the presence of either PBMC or 2H7scFv-Ig alone.

EXAMPLE 3

EFFECT OF SIMULTANEOUS LIGATION OF CD20 AND CD40 ON GROWTH OF NORMAL B CELLS, AND ON CD95 EXPRESSION, AND INDUCTION OF APOPTOSIS

This Example illustrates the effect on cell proliferation of cross-linking of CD20 and CD40 expressed on the cell surface.

Dense resting B cells were isolated from human tonsil by a Percoll step gradient and T cells were removed by E-rosetting. Proliferation of resting, dense tonsillar B cells was measured by uptake of ^3H -thymidine during the last 12 hours of a 4-day experiment. Proliferation was measured in quadruplicate cultures with means and standard deviations as shown. Murine anti-human CD20 monoclonal antibody 1F5 (anti-CD20) was used alone or was cross-linked with anti-murine κ monoclonal antibody 187.1 (anti-CD20XL). CD40 activation was accomplished using soluble human CD154 fused with murine CD8 (CD154) (Hollenbaugh *et al.*, *EMBO J.* 11: 4212-21 (1992)), and CD40 cross-linking was accomplished using anti-murine CD8 monoclonal antibody 53-6 (CD154XL). This procedure allowed simultaneous cross-linking of CD20 and CD40 on the cell surface. The results are presented in Figure 5.

The effect of CD20 and CD40 cross-linking on Ramos cells, a B lymphoma cell line, was examined. Ramos cells were analyzed for CD95 (Fas) expression and percent apoptosis eighteen hours after treatment (no goat anti-mouse IgG (GAM)) and/or cross-linking (+GAM) using murine monoclonal antibodies that specifically bind CD20 (1F5) and CD40 (G28-5). Control cells were treated with a non-binding isotype control (64.1) specific for CD3.

Treated Ramos cells were harvested, incubated with FITC-anti-CD95, and analyzed by flow cytometry to determine the relative expression level of Fas on the cell surface after CD20 or CD40 cross-linking. Data is plotted as mean fluorescence of cells after treatment with the stimuli indicated (Figure 6A).

Treated Ramos cells from the same experiment were harvested and binding of annexin V was measured to indicate the percentage apoptosis in the treated cultures. Apoptosis was measured by binding of Annexin V 18 hours after cross-linking of CD20 and CD40 using 1F5 and G28-5 followed by cross-linking with GAM. Binding of Annexin V was measured using a FITC-Annexin V kit (Catalog # PN-IM2376, Immunotech, Marseille, France,). Annexin V binding is known to be an early event in progression of cells into apoptosis. Apoptosis, or programmed cell death, is a process characterized by a cascade of catabolic reactions leading to cell death by suicide. In the early phase of apoptosis, before cells change morphology and hydrolyze DNA, the integrity of the cell membrane is maintained but cells lose the asymmetry of their membrane phospholipids, exposing negatively charged phospholipids, such as

phosphatidylserine, at the cell surface. Annexin V, a calcium and phospholipid binding protein, binds preferentially and with high affinity to phosphatidylserine. Results demonstrating the effect of cross-linking both CD20 and CD40 on expression of the FAS receptor (CD95) are presented in Figure 6B. The effect of cross-linking of both CD20 and CD40 on Annexin V binding to cells is shown in Figure 6B.

EXAMPLE 4

CONSTRUCTION AND CHARACTERIZATION OF 2H7 ScFv-CD154 FUSION PROTEINS

To construct a molecule capable of binding to both CD20 and CD40, cDNA encoding the 2H7 scFv was fused with cDNA encoding CD154, the CD40 ligand. The 2H7 scFv cDNA encoded on the HindIII-BclI fragment was removed from the 2H7 scFvIg construct, and inserted into a pD18 vector along with a BamHI-XbaI cDNA fragment encoding the extracellular domain of human CD154. The extracellular domain is encoded at the carboxy terminus of CD154, similar to other type II membrane proteins.

The extracellular domain of human CD154 was PCR amplified using cDNA generated with random primers and RNA from human T lymphocytes activated with PHA (phytohemagglutinin). The primer sets included two different 5' or sense primers that created fusion junctions at two different positions within the extracellular domain of CD154. Two different fusion junctions were designed that resulted in a short or truncated form (form S4) including amino acids 108 (Glu)-261 (Leu) + (Glu), and a long or complete form (form L2) including amino acids 48 (Arg) -261 (Leu) + (Glu), of the extracellular domain of CD154, both constructed as BamHI-XbaI fragments. The sense primer that fuses the two different truncated extracellular domains to the 2H7scFv includes a BamHI site for cloning. The sense primer for the S4 form of the CD154 cDNA is designated SEQUENCE ID NO: 11 or CD154BAM108 and encodes a 34 mer with the following sequence : 5'-gtt gtc gga tcc aga aaa cag ctt tga aat gca a-3' , while the antisense primer is designated SEQUENCE ID NO: 12 or CD154XBA and encodes a 44 mer with the following sequence: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'.

The oligonucleotide primers used in amplifying the long form (L2) of the CD154 extracellular domain encoding amino acids 48 (Arg)-261 (Leu) + (Glu), were as follows: The sense primer designated CD154 BAM48 (SEQUENCE ID NO:13) encoded a 35-mer with the following sequence: 5'-gtt gtc gga tcc aag aag gtt gga caa gat aga ag-3'. The antisense primer designated or CD154XBA (SEQUENCE ID NO:__) encoded the 44-

mer: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'. Other PCR reaction conditions were identical to those used for amplifying the 2H7 scFv (see Example 1). PCR fragments were purified by PCR quick kits (QIAGEN, San Diego, CA), eluted in 30 µl ddH₂O, and digested with BamHI and XbaI (Roche) restriction endonucleases in a 40 µl reaction volume at 37 °C for 3 hours. Fragments were gel purified, purified using QIAEX kits according to the manufacturer's instructions (QIAGEN), and ligated along with the 2H7 HindIII-BclI fragment into the pD18 expression vector digested with HindIII+XbaI. Ligation reactions were transformed into DH5-alpha chemically competent bacteria and plated onto LB plates containing 100 µg/ml ampicillin. Transformants were grown overnight at 37 °C, and isolated colonies used to inoculate 3 ml liquid cultures in Luria Broth containing 100 µg/ml ampicillin. Clones were screened after mini-plasmid preparations (QIAGEN) for insertion of both the 2H7 scFv and the CD154 extracellular domain fragments.

The 2H7scFv-CD154 construct cDNAs were subjected to cycle sequencing on a PE 9700 thermocycler using a 25-cycle program that included denaturing at 96 °C, 10 seconds, annealing at 50 °C for 5 seconds, and extension at 60°C, for 4 minutes. The sequencing primers used were pD18 forward (SEQ ID NO:__: 5'-gtctatataagcagagctctggc-3') and pD18 reverse (SEQ ID NO:__: 5'-cgaggctgatcagcgagctctagca-3') primers. In addition, an internal primer was used that had homology to the human CD154 sequence (SEQ ID NO:__: 5'-ccgcaatttgaggattctgacacc-3'). Sequencing reactions included primers at 3.2 pmol, approximately 200 ng DNA template, and 8 µl sequencing mix. Sequencing reactions were performed using the Big Dye Terminator Ready Sequencing Mix (PE-Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Samples were subsequently purified using Centrisep columns (Princeton Separations, Adelphia, NJ). The eluates were dried in a Savant speed-vacuum dryer, denatured in 20 µl template Suppression Reagent (ABI) at 95°C for 2 minutes, and analyzed on an ABI 310 Genetic Analyzer (PE-Applied Biosystems). The sequence was edited, translated, and analyzed using Vector Nti version 6.0 (Informax, North Bethesda, MD). The 2H7scFv-CD154 L2 cDNA sequence and predicted amino acid sequence is presented in Figure 7A, and 2H7scFv-CD154 S4 cDNA sequence and predicted amino acid sequence is presented in Figure 7B.

The binding activity of the 2H7 scFv-CD154 fusion proteins (SEQ. ID NO: ___ and ___) to CD20 and CD40 simultaneously was determined by flow cytometry. The assay used CHO cell targets that express CD20. After a 45-minute incubation of CD20 CHO cells with supernatants from cells transfected with the 2H7 scFv-CD154 expression
5 plasmid, the CD20 CHO cells were washed twice and incubated with biotin-conjugated CD40-Ig fusion protein in PBS/2% FBS. After 45 min, cells were washed twice and incubated with phycoerythrin (PE)-labeled streptavidin at 1:100 in PBS/2% FBS (Molecular Probes, Eugene OR). After an additional 30 min incubation, cells were washed 2X and were analyzed by flow cytometry. The results show that the 2H7 scFv-CD154 molecule
10 was able to bind to CD20 on the cell surface and to capture biotin-conjugated CD40 from solution (Figure 8).

To determine the effect of the 2H7scFv-CD154 on growth and viability of B lymphoma and lymphoblastoid cell lines, cells were incubated with 2H7scFv-CD154 L2 (SEQ. ID NO: ___) for 12 hours and then examined for binding of Annexin V. Binding of
15 Annexin V was measured using a FITC-Annexin V kit (Immunotech, Marseille, France, Catalog # PN-IM2376). B cell lines were incubated in 1 ml cultures with dilutions of concentrated, dialyzed supernatants from cells expressing secreted forms of the 2H7scFv-CD154 fusion proteins. The results are presented in Figure 9.

The growth rate of the Ramos B lymphoma cell line in the presence of
20 2H7scFv-CD154 was examined by uptake of ³H-thymidine for the last 6 hours of a 24-hour culture. The effect of 2H7scFv-CD154 on cell proliferation is shown in Figure 10.

EXAMPLE 5

CONSTRUCTION AND CHARACTERIZATION OF CYTOXB ANTIBODY DERIVATIVES

CytobX antibodies were prepared using 2H7 scFv-IgG polypeptide. The
25 2H7 scFv (see Example 1) was linked to the human IgG1 Fc domain via an altered hinge domain (see Figure 11). Cysteine residues in the hinge region were substituted with serine residues by site-directed mutagenesis and other methods known in the art. The mutant hinge was fused either to a wild-type Fc domain to create one construct, designated CytobX-MHWTG1C, or was fused to a mutated Fc domain (CytobX-MHMG1C) that had
30 additional mutations introduced into the CH2 domain. Amino acid residues in CH2 that are implicated in effector function are illustrated in Figure 11. Mutations of one or more of these residues may reduce FcR binding and mediation of effector functions. In this

Example, the leucine residue 234 known in the art to be important to Fc receptor binding, was mutated in the 2H7 scFv fusion protein, CytoxB-[MG1H/MG1C]. In another construct, the human IgG1 hinge region was substituted with a portion of the human IgA hinge, which was fused to wild-type human Fc domain (CytoxB-IgAHWTHG1C). (See Figure 11). This mutated hinge region allows expression of a mixture of monomeric and dimeric molecules that retain functional properties of the human IgG1 CH2 and CH3 domains. Synthetic, recombinant cDNA expression cassettes for these molecules were constructed and polypeptides were expressed in CHODG44 cells according to methods described in Example 2.

Purified fusion protein derivatives of CytoxB-scFvIg molecules were analyzed by SDS-PAGE according to the methods described in Example 2. Polyacrylamide gels were run under non-reducing and reducing conditions. Two different molecule weight marker sets, BioRad prestained markers, (BioRad, Hercules, CA) and Novex Multimark molecular weight markers were loaded onto each gel. The migration patterns of the different constructs and of Rituximab™ are presented in Figure 12.

The ability of the different derivatives of CytoxB-scFvIg molecules to mediate ADCC was measured using the Bjab B lymphoma cells as the target and freshly prepared human PBMCs as effector cells. (See Example 2). Effector to target ratios were varied as follows: 70:1, 35:1, and 18:1, with the number of Bjab cells per well remaining constant but the number of PBMCs were varied. Bjab cells were labeled for 2 hours with ^{51}Cr and aliquoted at a cell density of 5×10^4 cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or rituximab were added at a concentration of 10 $\mu\text{g/ml}$ to the various dilutions of PBMCs. Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 4 hours, and 100 μl of culture supernatant was harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released. The results are presented in Figure 13.

Complement dependent cytotoxicity (CDC) activity of the CytoxB derivatives was also measured. Reactions were performed essentially as described in Example 2. The results are presented in Figure 14 as percent of dead cells to total cells for each concentration of fusion protein.

EXAMPLE 6

IN VIVO STUDIES IN MACAQUES

Initial *in vivo* studies with CytoxB derivatives have been performed in nonhuman primates. Figure 15 shows data characterizing the serum half-life of CytoxB in monkeys. Measurements were performed on serum samples obtained from two different macaques (J99231 and K99334) after doses of 6 mg/kg were administered to each monkey on the days indicated by arrows. For each sample, the level of 2H7scFvIg present was estimated by comparison to a standard curve generated by binding of purified CytoxB-(MHWTG1C)-Ig fusion protein to CD20 CHO cells (see Example 2). The data are tabulated in the bottom panel of the Figure 15.

The effect of CytoxB-(MHWTG1C)Ig fusion protein on levels of circulating CD40+ cells in macaques was investigated. Complete blood counts were performed at each of the days indicated in Figure 16. In addition, FACS (fluorescence activated cell sorter) assays were performed on peripheral blood lymphocytes using a CD40-specific fluorescein conjugated antibody to detect B cells among the cell population. The percentage of positive cells was then used to calculate the number of B cells in the original samples. The data are graphed as thousands of B cells per microliter of blood measured at the days indicated after injection (Figure 16).

EXAMPLE 7

CONSTRUCTION AND EXPRESSION OF AN ANTI-CD19 scFv-IG FUSION PROTEIN

An anti-CD19 scFv-Ig fusion protein was constructed, transfected into eukaryotic cells, and expressed according to methods presented in Examples 1, 2, and 5 and standard in the art. The variable heavy chain regions and variable light chain regions were cloned from RNA isolated from hybridoma cells producing antibody HD37, which specifically binds to CD19. Expression levels of a HD37scFv-IgAHWTG1C and a HD37scFv-IgMHWTG1C were measured and compared to a standard curve generated using purified HD37 scFvIg. The results are presented in Figure 17.

EXAMPLE 8

CONSTRUCTION AND EXPRESSION OF AN ANTI-L6 scFv-IG FUSION PROTEIN

An scFv-Ig fusion protein was constructed using variable regions derived from an anti-carcinoma monoclonal antibody, L6. The fusion protein was constructed, transfected into eukaryotic cells, and expressed according to methods presented in

Examples 1, 2, and 5 and standard in the art. Expression levels of L6 scFv-IgAH WCH2 CH3 and L6 scFv-(SSS-S)H WCH2 WCH3 were measured and compared to a standard curve generated using purified L6 scFvIg. The results are presented in Figure 18.

EXAMPLE 9

CHARACTERIZATION OF VARIOUS scFv-IG FUSION PROTEINS

In addition to the scFv-Ig fusion protein already described, G28-1 (anti-CD37) scFv-Ig fusion proteins were prepared essentially as described in Examples 1 and 5. The variable regions of the heavy and light chains were cloned according to methods known in the art. ADCC activity of 2H7-MHWTG1C, 2H7-IgAHWTG1C, G28-1-MHWTG1C, G28-1 IgAHWTG1C, HD37- MHWTG1C, and HD37- IgAHWTG1C was determined according to methods described above (see Example 2). Results are presented in Figure 19. ADCC activity of L6scFv-IgAHWTG1C and L6scFv-IgMHWTG1C was measured using the 2981 human lung carcinoma cell line. The results are presented in Figure 20. The murine L6 monoclonal antibody is known not to exhibit ADCC activity.

The purified proteins were analyzed by SDS-PAGE under reducing and non-reducing conditions. Samples were prepared and gels run essentially as described in Examples 2 and 5. The results for the L6 and 2H7 scFv-Ig fusion proteins are presented in Figure 21 and the results for the G28-1 and HD37 scFv-Ig fusion proteins are presented in Figure 22.

EXAMPLE 10

CONSTRUCTION AND EXPRESSION OF ANTI-CD20 scFv-LLAMA Ig FUSION PROTEINS

This Example illustrates the cloning of llama IgG1, IgG2, and IgG3 constant region domains and the construction of immunoglobulin fusion proteins with each of the three constant regions and anti-CD20 scFv.

The constant regions of llama IgG1, IgG2, and IgG3 immunoglobulins were cloned and inserted into mammalian vector constructs containing an anti-CD20 single chain Fv, 2H7 scFv. Total RNA was isolated from peripheral blood mononuclear cells (PBMC) from llama blood (Triple J Farms, Bellingham, WA) by lysing the lymphocytes in TRIzol® (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. One microgram (1 µg) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA and 200 ng random primers were combined and

denatured at 72 °C for 10 minutes prior to addition of enzyme. Superscript II reverse transcriptase (Invitrogen Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 µl in the presence of 5X second strand buffer and 0.1 M DTT provided with the enzyme. The reverse transcription reaction was allowed to proceed at 42 °C for one hour. The cDNA was amplified by PCR using sequence specific primers. The 5' primers were designed according to published sequences for the V_{HH} and V_H domains of camelids. The 3' primer, which was used to amplify all three isotypes, was designed using mammalian CH3 domain sequences as a guide. The following specific primers were used. The Bcl and XbaI sites are indicated by underlined italicized sequences.

- 10 5' primer for llama IgG1 constant region
 LLG1-5'bgl: 5'-gtt gtt gat caa gaa cca cat gga gga tgc acg tg-3'
 (SEQ ID NO:___)
- 5' primer for llama IgG2 constant region
 LLG2-5'bgl: 5'-gtt gtt gat caa gaa ccc aag aca cca aaa cc-3'
 15 (SEQ ID NO:___)
- 5' primer for llama IgG3 constant region
 LLG3-5'bgl: 5'-gtt gtt gat caa gcg cac cac agc gaa gac ccc-3'
 (SEQ ID NO:___)
- 3' primer for llama IgG1, IgG2, and IgG3 constant regions
 20 LLG123-3'X: 5'-gtt gtt tct aga tta cta ttt acc cga aga ctg ggt gat gga-3'
 (SEQ ID NO:___)

PCR fragments of the expected size were cloned into TOPO® cloning vectors (Invitrogen Life Technologies) and then were sequenced. The sense sequencing primer, LLseqsense, had the sequence 5'-ctg aga tcg agt tca gct g-3' (SEQ ID NO:___), and
 25 the antisense primer, LLseqAS, had the sequence 5'-cct cct ttg gct ttg tct c-3' (SEQ ID NO:___). Sequencing was performed as described in Example 1. Figure 23 compares the amino acid sequence of the three isotype llama constant regions containing the hinge, CH2, and CH3 domains with the amino acid sequence of human IgG1 hinge, CH2, and CH3 domains.

30 After verifying the sequence, the amplified PCR products were digested with restriction enzymes BclI and XbaI to create compatible restriction sites. The digested fragments were then gel-purified, and the DNA was eluted using a QIAquick Gel

Extraction Kit (QIAGEN, Valencia, CA). The 2H7scFv-Ig pD18 mammalian expression vector construct (see Example 2) was digested with BclI and XbaI to remove the human IgG hinge, CH2, and CH3 domains. The pD18 vector is a modified derivative of pCDNA3 that contains an attenuated DHFR gene, which serves as a selectable marker for mammalian expression (Hayden *et al.*, *Tissue Antigens* 48:242-54 (1996)). The purified llama IgG1, IgG2, and IgG3 constant region PCR products were ligated by T4 DNA ligase (Roche Molecular Biochemicals, Indianapolis, IN) into the double-digested 2H7 scFv-pD18 vector at room temperature overnight according to the manufacturer's instructions. After ligation, the ligation products were transformed into *E. coli* DH5 α bacteria (BD Biosciences, Palo Alto, CA) and plated according to standard molecular biology procedures and manufacturer's instructions. Isolated colonies were chosen to screen for transformants containing the correct inserts.

For expression of the encoded polypeptides, plasmid DNA from positive clones was transiently transfected into COS-7 cells using DEAE-dextran (Hayden *et al.*, *Ther Immunol.* 1:3-15 (1994)). COS-7 cells were seeded at approximately 3×10^6 cells per 150 mm plate and grown overnight until the cells were about 75% confluent. Cells were then washed once with serum-free DMEM (Invitrogen Life Technologies, Grand Island, NY). Transfection supernatant (10 ml) containing 400 μ g/ml DEAE-dextran, 0.1 mM chloroquine, and 5 μ g/ml of the DNA constructs were added to the cells, which were then incubated at 37 °C for 3-4 hrs. After incubation, cells were pulsed with 10 ml of 10% dimethyl sulfoxide (DMSO) in 1x PBS at room temperature for 2 minutes. Cells were then placed back into fully supplemented DMEM/10% FBS (1% L-glutamine, 1% penicillin/streptomycin, 1% sodium pyruvate, 1% MEM essential amino acids) (Invitrogen Life Technologies). After 24 hours, the media was replaced with serum-free fully supplemented DMEM (Invitrogen Life Technologies), and the cells were maintained up to 21 days with media changes every 3-4 days.

Ig-fusion proteins were purified by passing COS cell culture supernatants through Protein A Agarose (Repligen, Cambridge, MA) columns. After application of the culture supernatant, the Protein A columns were then washed with 1x PBS (Invitrogen Life Technologies). Bound Ig-fusion proteins were eluted with 0.1 M citric acid (pH 2.8), and the collected fractions were immediately neutralized with Tris base (pH 10.85). The fractions containing protein were identified by measuring the optical density (A_{280}) and

then were pooled, dialyzed against 1x PBS, (Invitrogen Life Technologies) and filtered through a 0.2 μ m filter.

The purified Ig-fusion proteins were analyzed by SDS-PAGE. Aliquots of 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, and Rituxan® (Rituximab, anti-CD20 antibody, Genentech, Inc. and IDEC Pharmaceuticals Corp.) (5 μ g protein) were combined with 25 μ l 2x NuPAGE® SDS Sample Buffer (Invitrogen Life Technologies) (non-reduced samples). Samples of each protein were also prepared in reducing sample buffer containing 5% 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO). Molecular weight markers (Invitrogen Life Technologies) were applied to the gels in non-reducing buffer only. The proteins were fractionated on NuPAGE® 10% Bis-Tris gels (Invitrogen Life Technologies). After electrophoresis (approximately 1 hour), the gels were washed three times, five minutes each, with Distilled Water (Invitrogen Life Technologies) and then stained in 50 ml Bio-Safe Coomassie Stain (BioRad, Hercules, CA) overnight at room temperature. After a wash in Distilled Water, the gels were photographed. The migration pattern of each Ig-fusion protein is presented in Figure 24.

The ability of the 2H7 scFv-llama Ig fusion proteins to bind to cells expressing CD20 was demonstrated by flow cytometry. Serial dilutions starting at 25 μ g/ml of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, and 2H7 scFv-llama IgG3 were prepared and incubated with CD20-transfected (CD20+) CHO cells (from the laboratory of Dr. S. Skov, Institute of Medical Microbiology and Immunology, Copenhagen Denmark in 1% FBS 1x PBS media (Invitrogen Life Technologies) for one hour on ice. After the incubation, the cells were then centrifuged and washed with 1% FBS in 1x PBS. To detect bound 2H7 scFv-llama Ig, the cells were incubated for one hour on ice with fluorescein-conjugated goat anti-camelid IgG (heavy and light chain) (1:100) (Triple J Farms). The cells were then centrifuged and resuspended in 1% FBS-1x PBS and analyzed using a Coulter Epics XL cell sorter (Beckman Coulter, Miami, FL). The data (percent of maximum brightness) are presented in Figure 25.

EXAMPLE 11

EFFECTOR FUNCTION OF ANTI-CD20 SCFV-LLAMA IG FUSION PROTEINS

This Example demonstrates the ability of anti-CD20 llama IgG1, IgG2, and IgG3 fusion proteins to mediate complement dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC).

The ability of the 2H7 scFv-llama IgG fusion proteins to kill CD20 positive cells in the presence of complement was tested using the BJAB human B cell line. Rabbit complement was obtained from 3-4 week old rabbits (Pel-Freez, Brown Deer, WI). BJAB cells (2×10^6 cells/ml) were combined with rabbit complement (final dilution 1:10) and purified 2H7 Ig fusion proteins. 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, and 2H7 scFv-human IgG1 wild type hinge-CH2-CH3 (Example 1) were added at 1:3 serial dilutions beginning at a concentration of 30 μ g/ml. After one hour at 37 °C, cell viability was determined by counting live and dead cells by trypan blue exclusion (0.4%) (Invitrogen Life Technologies) using a hemacytometer (Bright-line, Horsham, PA). The percent killing was calculated by dividing the number of dead cells by the number of total cells (dead + live cells). The data presented in Figure 26 show that all Ig fusion proteins had CDC activity.

The ADCC activity of the 2H7 scFv-llama IgG fusion proteins was determined using BJAB cells as target cells and human or llama peripheral blood mononuclear cells (PBMC) as effector cells. BJAB cells were pre-incubated for approximately 2 hours with ^{51}Cr (100 μ Ci) (Amersham Biosciences, Piscataway, NJ) in fully supplemented IMDM (Invitrogen Life Technologies) containing 15% FBS. The cells were mixed intermittently during the pre-incubation period. Fresh, resting human PBMC were purified from whole blood using Lymphocyte Separation Media (LSM) (ICN Pharmaceuticals, New York, NY). PBMC were combined with labeled BJAB cells (5×10^4 cells per well of 96 well tissue culture plate) at ratios of 25:1, 50:1, and 100:1. To each combination was added 10 μ g/ml of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, Rituximab, or no anti-CD20 antibody. The mixtures were incubated for 6 hours at 37 °C. Supernatant from each reaction containing ^{51}Cr released from lysed cells was collected onto a LumaPlate-96 filter plate (Packard, Meriden, CT), which was dried overnight. The amount of ^{51}Cr was measured by a TopCount NXT plate reader (Packard). Figure 27 shows that the 2H7 scFv-llama IgG2 fusion protein was the most effective llama fusion protein in mediating ADCC. Each data point represents the average measurement of triplicate wells.

ADCC activity was affected by the source of effector cells. Llama PBMC were isolated from llama blood (Triple J Farms) using LSM. Llama effector cells were added at the same ratios to BJAB target cells as described for the ADCC assay using

human effector cells. The cells were combined with 10 µg/ml of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, Rituximab, or no anti-CD20 antibody. The results are presented in Figure 28.

EXAMPLE 12

CONSTRUCTION AND CHARACTERIZATION OF SCFV IG FUSION PROTEINS EXPRESSED ON THE CELL SURFACE

This Example describes a retroviral transfection system for ectopic surface expression of genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors. The Example also demonstrates the effector function of these various scFv Ig fusion proteins expressed on the surface of target cells.

The heavy and light chain variable regions were cloned from murine monoclonal antibodies specific for various costimulatory receptors, and single chain Fv constructs were prepared essentially as described in Example 1. Antibodies included 2H7, anti-human CD20; 40.2.220, anti-human CD40; 2E12, anti-human CD28; 10A8, anti-human CD152 (anti-CTLA-4); and 500A2, anti-murine CD3. The heavy chain and light chain variable regions of each antibody were cloned according to standard methods for cloning immunoglobulin genes and as described in Example 1. Single chain Fv constructs were prepared as described in Example 1 by inserting a nucleotide sequence encoding a (gly₄ser)₃ peptide linker between the VL region nucleotide sequence of 40.2.220, 2E12, 10A8, and 500A2, respectively (SEQ ID NOs: __, respectively) and the VH region nucleotide sequence of 40.2.220, 2E12, 10A8, and 500A2, respectively (SEQ ID NOs: __, respectively). The polypeptide sequence for VL of 40.2.220, 2E12, 10A8, and 500A2 are set forth in SEQ ID NOs: __, respectively, and the polypeptide sequence for VH of 40.2.220, 2E12, 10A8, and 500A2 are set forth in SEQ ID NOs: __, respectively. Each scFv polynucleotide (SEQ ID NOs: __ for 40.2.220, 2E12, 10A8, and 500A2, respectively) was then fused to human IgG1 mutant hinge (CCC→SSS) and mutant CH2 (proline to serine mutation at residue 238 (238 numbering according to EU nomenclature, Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94; residue 251 according to Kabat *et al.*) and wild type CH3 domains according to the methods described in Example 5 and 11. Each scFv mutant IgG1 fusion polynucleotide sequence was then fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO: __), such that when the fusion protein was expressed in the transfected cell, CD80 provided an anchor for

surface expression of the scFv Ig fusion protein. cDNAs encoding the scFv-IgG-CD80 fusion proteins (SEQ ID NOs: __ for 40.2.220-, 2E12-, 10A8-, and 500A2-scFv-IgG-CD80, respectively) were inserted into the retroviral vector pLNCX (BD Biosciences Clontech, Palo Alto, CA) according to standard molecular biology procedures and vendor instructions. The scFv-Ig-CD80 cDNA was inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence. The retroviral constructs were transfected into Reh, an acute lymphocytic leukemia cell line (ATCC CRL-8286). Transfected cells were screened to select clones that were expressing scFv-Ig fusion proteins on the cell surface.

CDC and ADCC assays were performed with the transfected Reh cells to determine if expression of the scFv-Ig polypeptides on the cell surface augmented effector cell function. Reh cells expressing anti-human CD152 scFv-mutant IgG-CD80 (SEQ ID NO:__); Reh anti-human CD28 scFv- mutant IgG-CD80 (SEQ ID NO:__); Reh anti-human CD28 scFv- mutant IgG-CD80 (SEQ ID NO:__); Reh anti-human CD40 scFv-mutant IgG-CD80 (SEQ ID NO:__); Reh anti-human CD20 scFv- mutant IgG-CD80 (SEQ ID NO:__) were combined with human PBMC (see Example 11) and rabbit complement (10 µg/ml) for one hour at 37 °C. Untransfected Reh cells were included as a control. Viability of the cells was determined by trypan blue exclusion, and the percent of killed cells was calculated (see Example 11). Figure 29 shows the effectiveness of the scFv-IgG-CD80 fusion proteins when expressed on the cell surface of tumor cells to mediate complement dependent cytotoxicity.

The same transfected Reh cells tested in the CDC assay plus Reh cells transfected with the polynucleotide construct that encodes anti-murine CD3-scFv-Ig-CD80 (SEQ ID NO:__) were analyzed for ADCC activity (see Example 11). Untransfected and transfected Reh cells were pre-labeled with ⁵¹Cr (100 µCi) (Amersham) for two hours at 37 °C. Human PBMC served as effector cells and were added to the Reh target cells (5 x 10⁴ cells per well of 96 well plate) at ratios of 5:1, 2.5:1, and 1.25:1. After five hours at 37 °C, culture supernatants were harvested and analyzed as described in Example 11. Percent specific killing was calculated according to the following equation: ((experiment release minus spontaneous release)/(maximum release minus spontaneous release)) x 100. The data are presented in Figure 30. Each data point represents the average of quadruplicate samples.

Using the same procedures described above, the same results with other binding domains were obtained using the following monoclonal antibodies monoclonal antibodies as sources of sFv: for CD20, 1F5 (Genbank AY 058907 and AY058906); for CD40, 2.36 and G28.5; for CD28, 9.3.

5 Cell surface expression of antibody binding domains is accomplished by fusing antibody scFvs to IgA hinge and constant regions and IgE hinge-acting region, *i.e.*, IgE CH2, and constant regions. Polynucleotides encoding an anti-4-1BB scFv, 5B9 (anti-human 4-1BB) scFv, and 2e12 (anti-human CD40) fused to IgAH IgA T4 (four terminal CH3 residues deleted) fused to the CD80 transmembrane and cytoplasmic domains and IgE
10 Fc regions are shown in SEQ ID NOs:____. The encoded polypeptides are shown in SEQ ID NOs:____.

EXAMPLE 13

CONSTRUCTION AND SEQUENCE OF HUMAN IG HINGE-CH2-CH3 MUTANTS AND 2H7

VARIABLE REGION MUTANTS

15 This Example describes construction of scFv fusion proteins containing mutant human IgG1 and IgA constant regions. This Example also describes construction of a 2H7 scFv mutant with a single point mutation in the variable heavy chain region. Mutations were introduced into variable and constant region domains according to methods described herein and known in the molecular biology arts. Figure 31 presents
20 nomenclature for the Ig constant region constructs.

The human IgG1 hinge region of the 2H7 scFv human IgG1 hinge-CH2-CH3 fusion proteins was mutated to substitute cysteine residues that in a whole immunoglobulin are involved in forming disulfide bonds between two heavy chain molecules. One mutant, 2H7 scFv fused to a human IgG1 hinge region in which all three
25 cysteine residues were mutated to serine residues (MTH (SSS)), was prepared as described in Example 5 (designated in Example 5 as CytoxB-MHWTG1C (includes wild type IgG1 CH2 and CH3 domains)) (now referred to as 2H7 scFv MTH (SSS) WTCH2CH3) and comprises the polynucleotide sequence SEQ ID NO:____ encoding the polypeptide as set forth in SEQ ID NO____. The polynucleotide sequence encoding this mutant (SEQ ID
30 NO:____) was used as a template to create mutant hinge regions in which the first two cysteine residues were substituted with serine residues (IgG MTH (SSC)). An oligonucleotide was designed to substitute the third serine residue with a cysteine and had

the following sequence: 5'-gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tgc cca gca cct g-3' (HuIgGMHncs3, SEQ ID NO:___). A second mutant was prepared in which the mutant hinge had serine residues substituting the first and third cysteine residues (IgG MTH (SCS)). The sequence of the oligonucleotide to create this mutant was as follows: 5'-gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tgc cca ccg-3' (HuIgGMHncs2, SEQ ID NO:___). A third mutant was prepared with cysteine residues substituted at the second and third positions (IgG MTH (CSS)), also using the IgG MTH (SSS) mutant as template, and an oligonucleotide having the sequence, 5'-gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac-3' (HuIgGMHncs1, SEQ ID NO:___).

The oligonucleotides introducing the mutations into the hinge region were combined with template and a 3' oligonucleotide containing an XbaI site (underlined and italicized) (5'-gtt gtt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g-3' (SEQ ID NO:___)) to amplify the mutant hinge-wild type (WT)-CH2-CH3 sequences by PCR. The IgG MTH CSS and IgG MTH SCS mutant sequences were amplified for 25 cycles with a denaturation profile of 94 °C, annealing at 52 °C for 30 seconds, and extension at 72 °C for 30 seconds. The IgG MTH SSC mutant sequence was amplified under slightly different conditions: denaturation profile of 94 °C, annealing at 45 °C for 30 seconds, and extension at 72 °C for 45 seconds. The amplified polynucleotides were inserted into the TOPO® cloning vector (Invitrogen Life Technologies) and then were sequenced as described in Example 1 to confirm the presence of the mutation. pD18 vector containing 2H7 scFv was digested to remove the constant region sequences essentially as described in Example 10. The mutant hinge-wild type CH2-CH3 regions were inserted in frame into the digested vector DNA to obtain vectors comprising 2H7 scFv MTH (CSS) WTCH2CH3 encoding DNA (SEQ ID NO:___); 2H7 scFv MTH (SCS) WTCH2CH3 encoding DNA (SEQ ID NO:___); and 2H7 scFv MTH (SSC) WTCH2CH3 encoding DNA (SEQ ID NO:___).

A mutation of leucine to serine at position 11 in the first framework region of the heavy chain variable region (numbering according to Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)) was introduced into the 2H7 scFv MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO:___). The wild type leucine residue was substituted with serine by site-directed mutagenesis using the oligonucleotide Vhser11: 5'-gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc-3' (SEQ ID NO:___)(this sequence, or an

amino acid sequence that it encodes, may be optionally excluded from particular claimed embodiments of the instant invention). The 3'-primer for PCR was huIgG1-3' having the sequence 5'-gtc tct aga cta tca ttt acc cgg aga cag-3' (SEQ ID NO:___) (XbaI site underlined and italicized). After PCR amplification, the fragments were inserted into the

5 TOPO® cloning vector and sequenced to confirm the presence of the VH11 leucine to serine mutation. The 2H7 scFv-IgG MTH (SSS) WTCH2CH3 encoding DNA was shuttled into the PSL1180 cloning vector (Pharmacia Biotech, Inc., Piscataway, NJ). The construct PSL1180-2H7 scFv-IgG MTH (SSS) WTCH2CH3 was digested with Sac and XbaI to remove the wild type VH domain and the hinge and CH2 and CH3 domains. The

10 PCR product comprising the VH11 mutant was digested with Sac and XbaI and then inserted into the digested PSL1180 construct according to standard molecular biology procedures. The construct was then digested with Hind III and XbaI, and inserted into the mammalian expression vector pD18 (see methods described in Example 1 and Example 10). The mutant is designated 2H7 scFv VH11SER IgG MTH (SSS) WTCH2CH3 (Figure

15 31). The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded polypeptide sequence is provided in SEQ ID NO:___ (these sequences may be optionally excluded from particular claimed embodiments of the instant invention).

Four constructs containing IgA constant region domains were prepared. One construct contained wild type IgA hinge fused to human IgG1 CH2 and CH3 (IgAH

20 IgG WTCH2CH3) (Figure 31). Sequential PCR amplifications were performed to substitute the human IgG1 hinge of the 2H7 scFv construct with nucleotide sequences encoding the IgA hinge. The 5' oligonucleotide primer (huIgA/Gchim5) for the first PCR reaction had the sequence, 5'-cca tct ccc tca act cca cct acc cca tct ccc tca tgc gca cct gaa ctc ctg-3' (SEQ ID NO:___). The primer (huIgAhg-5') for the second PCR reaction to add

25 more IgA specific hinge sequence and add a BclI restriction enzyme site (italicized and underlined) had the sequence, 5'-gtt gtt gat cag cca gtl ccc tca act cca cct acc cca tct ccc caa ct-3' (SEQ ID NO:___). The 3' primer for both amplification steps was huIgG1-3' having the sequence, 5'-gtc tct aga cta tca ttt acc cgg aga cag-3' (SEQ ID NO:___). The sequence of the PCR product was confirmed by TOPO® cloning as described above. The

30 gel-purified fragment was digested with BclI and XbaI and then inserted into the 2H7 scFv-pD18 vector that had been digested BclI and XbaI to remove all the IgG1 constant region domains. Ligation was performed as described in Example 10 to provide a

mammalian expression vector comprising the nucleotide sequence (SEQ ID NO:___) encoding a 2H7 scFv IgA hinge-IgG1 CH2-CH3 polypeptide (SEQ ID NO:___).

A second pD18 mammalian expression vector was constructed that had a polynucleotide sequence (SEQ ID NO:___) that encoded a 2H7 scFv fused to wild type IgA hinge, CH2, and CH3 domains (SEQ ID NO:___). Human IgA constant regions sequences were obtained by using random primers to reverse transcribe total RNA isolated from human tonsil followed by PCR amplification of the cDNA using sequence specific primers, essentially as described in Example 10. Human IgA hinge-CH2-CH3 nucleotide sequence (SEQ ID NO:___) encoding the IgA-CH2-CH3 polypeptide (IgAH IgACH2CH3, Figure 31) (SEQ ID NO:___) was amplified using the 5' oligonucleotide huIgAhg-5' (SEQ ID NO:(*same as above*)___) and a 3' oligonucleotide huIgA3' having the sequence, 5'-gtt gtt tct aga tta tca gta gca ggt gcc gtc cac ctc cgc cat gac aac-3' (SEQ ID NO:___). Secretion of a 2H7-IgA hinge-IgA CH2-CH3 polypeptide from transfected mammalian cells required co-expression of human J chain that covalently binds to two IgA CH3 domains via disulfide bonds. Total RNA was isolated from tonsil B cells and was reversed transcribed to generate cDNA as described above. PCR amplification of the nucleotide sequence encoding the J chain was performed with J chain specific primers. The 5' PCR primer, HUIJCH5nl, had the sequence, 5'-gtt gtt aga tct caa gaa gat gaa agg att gtt ctt-3' (SEQ ID NO:___), and sequence of the 3' primer, HUIJCH3, was 5'-gtt gtt tct aga tta gtc agg ata gca ggc atc tgg-3' (SEQ ID NO:___). The cDNA was cloned into TOPO® for sequencing as described in Example 10. J chain encoding cDNA (SEQ ID NO:___) was then inserted into pD18 and pCDNA3-Hygro (+) (Invitrogen Life Technology) vectors for co-transfection with 2H7 scFv IgA hinge-CH2-CH3 constructs. The J chain has the predicted amino acid sequence set forth in SEQ ID NO:___.

Secretion of an scFv IgA constant region construct in the absence of J chain was accomplished by engineering a truncated CH3 domain with a deletion of the four carboxy terminal amino acids (GTCY, SEQ ID NO:___) (IgAH IgA-T4, Figure 31), which include a cysteine residue that forms a disulfide bond with the J chain. The IgA hinge-CH2-CH3 nucleotide sequence containing the deletion in CH3 (SEQ ID NO:___) was prepared using a 5' PCR primer (huIgAhg-5') having the sequence 5'-gtt gtt gat cag cca gtt ccc tca act cca cct acc cca tct ccc tca act-3' (SEQ ID NO:___) (BclI site is underlined and italicized), and a 3' PCR primer (HUIGA3T1) having the sequence 5'-gtt gtt tct aga tta tca

gtc cac ctc cgc cat gac aac aga cac-3' (SEQ ID NO:___). This mutated IgA constant region nucleotide sequence was inserted into a 2H7 scFv pD18 vector as described for the generation of the previous 2H7 scFv-Ig constructs (see Example 1 and this Example) that comprises the polynucleotide sequence (SEQ ID NO:___) encoding a 2H7 IgAH IgA-T4 polynucleotide (SEQ ID NO:___).

A fourth construct was prepared that encoded a 2H7 scFv-IgA constant region fusion protein with a deletion of 14 additional amino acids, most of which are hydrophobic residues, from the carboxy terminus of IgA CH3. The 2H7 scFv-IgAH IgA-T4 encoding polynucleotide was used as template to engineer a deletion of the nucleotide sequence encoding PTHVNVSVVMAEVD (SEQ ID NO:___). The 5' oligonucleotide primer had the sequence 5'-gtt gtt gat cag cca gtt ccc tca act cca cct acc cca tct ccc tca act-3' (SEQ ID NO:___) (BclI site shown as underlined and italicized). The 3' oligonucleotide sequence was 5'-gtt gtt tct aga tta tca ttt acc cgc caa gcg gtc gat ggt ctt-3' (SEQ ID NO:___). The deleted IgA CH3 region was amplified by using the above oligonucleotides to amplify the IgA constant region from RNA isolated from human tonsil such that the cDNA contained the deleted carboxyl terminal encoding region for the 18 amino acids. The IgAH IgA-T18 constant region was inserted into a 2H7 scFv pD18 vector that comprises the polynucleotide sequence (SEQ ID NO:___) encoding a 2H7 IgAH IgA-T18 polynucleotide (SEQ ID NO:___) as described above.

EXAMPLE 14

EFFECTOR FUNCTION OF CTLA-4 IGG FUSION PROTEINS

The Example compares the effector functions of CTLA-4 Ig fusion proteins in CDC and ADCC assays.

Two CTLA-4 IgG fusion proteins were constructed. One fusion protein comprises the extracellular domain of CTLA-4 fused to human IgG1 wild type hinge, CH2, and CH3 domains and is designated CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:___). A pD18 mammalian expression vector comprising a polynucleotide sequence encoding CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:___) was prepared by fusing in frame the nucleotide sequence encoding the extracellular domain of CTLA-4 (SEQ ID NO:___) (see U.S. Patent No. 5,844,095) to the nucleotide sequence encoding IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:___) according to the methods described in Examples 1 and 10. The extracellular domain nucleotide sequence also comprises a BclI

restriction enzyme site at the 3' end, and a leader peptide nucleotide sequence (SEQ ID NO:___) that encodes an oncoM leader peptide (SEQ ID NO:___). A second CTLA-4 IgG fusion protein, designated CTLA-4 IgG MTH (SSS) MTCH2WTCH3, contained the extracellular domain of CTLA-4 (plus the oncoM leader peptide sequence) fused to a mutant IgG hinge in which all three cysteine residues were replaced with serine residues. The hinge region was fused to a mutant IgG1 CH2 domain that had a mutation at isotype position 238 (EU numbering, Ward *et al.*, *supra*, (position 251 using numbering according to Kabat *et al.*, *supra*; position 209 where numbering commences with first residue of IgG1 CH1; *i.e.*, PAPELLDGPS (SEQ ID NO:___) of wild type IgG1 CH2 is modified to PAPELLDGSS (SEQ ID NO:___)), which was fused to IgG1 wild type CH3 (U.S. Patent No. 5,844,095). The CTLA-4 IgG MTH (SSS) MTCH2WTCH3 polynucleotide comprises the nucleotide sequence in SEQ ID NO:___ and the deduced amino acid sequence comprises the sequence provided in SEQ ID NO:___.

CTLA-4 fusion proteins were also prepared using CTLA-4 extracellular membrane encoding sequences without the leader peptide (SEQ ID NO:___).

To measure CDC activity, purified CTLA-4 IgG WTH (CCC) WTCH2CH3 (2 µg/ml) or CTLA-4 IgG MTH (SSS) MTCH2WTCH3 (2 µg/ml) was added to Reh cells (see Example 12) and to Reh cells transfected with the costimulatory molecule CD80 such that CD80 was expressed on the cell surface (Reh CD80.10; see Doty *et al.*, 1998 *J. Immunol.* 161:2700; Doty *et al.*, 1996 *J. Immunol.* 157:3270), in the presence or absence of rabbit complement (10 µg/ml). Purified CTLA Ig fusion proteins were prepared from culture supernatants of transiently transfected COS cells according to methods described in Example 10. The assays were performed essentially as described in Example 11 and 12. The data presented in Figure 32 show that only CD80-transfected Reh cells were killed in the presence of complement and CTLA-4 IgG WTH (CCC) WTCH2CH3 fusion protein.

The purified CTLA-4 Ig fusion proteins were also tested in ADCC assays. Human PBMC, serving as effector cells, were added to Reh or Reh CD80.1 target cells at a ratio of 1.25:1, 2.5:1, 5.0:1, and 10:1. Cells were labeled and the assays performed essentially as described in Examples 11 and 12. The results are presented in Figure 33. Each data point represents the average of four independent culture wells at each effector:target cell ratio. The data show that only CTLA-4 IgG WTH (CCC) WTCH2CH3 mediated significant ADCC of Reh CD80.10 cells.

EXAMPLE 15**EFFECTOR FUNCTION OF CTLA-4 IGA FUSION PROTEINS**

CTLA-4 IgA fusion proteins are prepared as described for the IgG fusion proteins (see Examples 1, 13, and 14). CTLA-4 extracellular domain nucleotide sequence (SEQ ID NO:___) is fused in open reading frame to nucleotides encoding IgAH IgACH2CH3 (SEQ ID NO:___) to provide the nucleotide sequence (SEQ ID NO:___) encoding a CTLA-4 IgAH IgACH2CH3 fusion protein (SEQ ID NO:___). The fusion protein is transiently expressed in COS cells (see Example 10) or stably expressed in CHO cells (see Example 1). Secretion of the CTLA-4 IgAH IgACH2CH3 fusion protein requires co-transfection with a construct containing a polynucleotide sequence (SEQ ID NO:___) that encodes human J chain (SEQ ID NO:___). The CTLA-4 IgAH IgACH2CH3 fusion protein is isolated as described in Examples 10 and 14. To express a CTLA-4 IgA construct without the presence of J chain, a CTLA-4 IgAH IgA-T4 construct is prepared and transfected into mammalian cells. In a similar manner as described for the CTLA-4 extracellular fragment fused to wild type IgA hinge-CH2CH3, the CTLA-4 extracellular domain nucleotide sequence (SEQ ID NO:___) is fused in open reading frame to a nucleotide sequence (SEQ ID NO:___) encoding a IgAH IgA-T4 polypeptide (SEQ ID NO:___) to provide a nucleotide sequence comprising SEQ ID NO:___ encoding a CTLA-4 IgAH IgA-T4 polypeptide (SEQ ID NO:___). Effector function of each construct is evaluated by CDC and ADCC as described in Example 14.

EXAMPLE 16**BINDING OF ANTI-CD20 scFv HUMAN Ig FUSION PROTEINS****TO CHO CELLS EXPRESSING CD20**

This Example describes binding of 2H7 scFv Ig fusion proteins to CHO cells that express CD20. The analysis was performed by flow cytometry. Culture supernatants were collected from transiently transfected COS cells expressing 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:___); 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO:___); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:___); and 2H7 scFv VHSE11 WTH WTCH2CH3 (SEQ ID NO:___), and two-fold serial dilutions were prepared. Serial two-fold dilutions of purified 2H7 scFv IgG MTH (SSC) WTCH2CH3 (SEQ ID NO:___) were prepared starting at a concentration of 5 µg/ml. The culture supernatants and purified fusion protein samples were incubated with (CD20+) CHO cells

for one hour on ice. The cells were washed twice and then incubated with 1:100 FITC-conjugated goat anti-human IgG (CalTag) for 40 minutes. The unbound conjugate was then removed by washing the cells and flow cytometry analysis was performed using a Coulter Epics XL cell sorter. Results are presented in Figure 34.

EXAMPLE 17

IMMUNOBLOT ANALYSIS OF ANTI-CD20 scFv HUMAN IgG AND IGA FUSION PROTEINS

This Example describes immunoblot analysis of 2H7 scFv IgG and 2H7 scFv IgA fusion proteins that were immunoprecipitated from transfected cell culture supernatants.

COS cells were transiently transfected with plasmids comprising nucleotide sequences for 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgA H IgG WTCH2CH3 (SEQ ID NO:); and scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:) essentially according to the method described in Example 10. Cells were also transfected with vector only. After 48-72 hours at 37°C, cell culture supernatants were harvested and combined with protein A-agarose beads (Repligen) for one hour at 4 °C. The beads were centrifuged and washed several times in TNEN [20 mM Tris base, 100 mM NaCl, 1 mM EDTA, and 0.05% NP-40, pH 8.0). The immunoprecipitates were combined with 25 µl 2x NuPAGE® SDS Sample Buffer (Invitrogen Life Technologies) (non-reduced samples). The proteins were fractionated on NuPAGE® 10% Bis-Tris gels (Invitrogen Life Technologies). After electrophoresis (approximately 1 hour), the proteins were transferred from the gel onto a Immobilon P polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA) using a semi-dry blotter (Ellard Instrumentation, Monroe, WA). The PVDF membrane was blocked in PBS containing 5% nonfat milk and then probed with HRP-conjugated goat anti-human IgG (Fc specific) (CalTag). After washing the immunoblot several times in PBS, the blot was developed using ECL (Amersham Biosciences). The results are shown in Figure 35.

EXAMPLE 18

BINDING OF ANTI-CD20 scFv HUMAN IGA FUSION PROTEINS TO CD20+ CHO CELLS

This Example describes flow immunocytofluorimetry analysis of binding of 2H7 scFv IgAH IgACH2CH3 (SEQ ID NO:___) and 2H7 scFv IgAH IgAT4 (SEQ ID NO:___) fusion proteins to (CD20+) CHO cells.

COS cells were transiently co-transfected as described in Example 10 with
 5 plasmid DNA comprising a polynucleotide sequence (SEQ ID NO:___) encoding 2H7 scFv IgAH IgACH2CH3 polypeptide (SEQ ID NO:___) and with a separate plasmid comprising a polynucleotide sequence (SEQ ID NO:___) encoding a human J chain polypeptide (SEQ ID NO:___). COS cells were also transfected with a polynucleotide sequence (SEQ ID NO:___) encoding a 2H7 scFv IgA fusion protein that had a deletion of
 10 four amino acids at the carboxy terminus of CH3 (2H7 scFv IgAH IgA-T4, SEQ ID NO:___). The transfections were performed as described in Example 10. Culture supernatants from transfected COS cells were combined with (CD20+) CHO cells (see Example 1) and incubated for one hour on ice. The cells were washed twice with PBS-2%FBS and then combined with FITC-conjugated goat anti-human IgA chain (CalTag)
 15 (1:100) for 40 minutes. The cells were again washed and then analyzed by flow cytometry using a Coulter Epics XL cell sorter. Figure 36 shows that co-transfection with J chain was not required for secretion of 2H7 scFv IgAH IgAT4, the 2H7 IgA fusion protein with the truncated CH3 carboxy end (SEQ ID NO:___).

EXAMPLE 19

20 EFFECTOR FUNCTION OF ANTI-CD20 scFv HUMAN IGA FUSION PROTEINS

This Example illustrates ADCC activity of 2H7 IgG and IgA fusion proteins against cells that express CD20. BJAB cells were prelabeled with ⁵¹Cr (100 μCi) (Amersham) for two hours at 37 °C. Effector cells were obtained from fresh, resting human whole blood, which was diluted in an equal volume of Alsever's solution to prevent
 25 coagulation. 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:___); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:___); 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:___); and 2H7 scFv IgAH IgACH2CH3 (SEQ ID NO:___) fusion proteins were purified from transiently transfected COS cell supernatants (100-200 ml) by protein A chromatography as described in Example 10. COS cells transfected with the plasmid
 30 encoding 2H7 scFv IgAH IgACH2CH3 were co-transfected with a plasmid encoding human J chain as described in Example 18. Two-fold serial dilutions of the purified 2H7 Ig fusion proteins starting at 5 μg/ml were added to the labeled BJAB cells (5 x 10⁴ cells

per well of 96 well tissue culture plate) in the presence of whole blood (100 µl of whole blood diluted 1:1 in Alsever's solution, final dilution 1:4) and incubated for five hours at 37 °C. Culture supernatants were harvested and analyzed as described in Example 11. Percent specific killing was calculated according to the following equation: ((experiment
 5 release minus spontaneous release)/(maximum release minus spontaneous release)) x 100. The data are presented in Figure 37. Each data point represents the average of quadruplicate samples.

In a second ADCC assay, the number of labeled BJAB target cells was held constant in each sample, and whole blood was added at dilutions of 0.25, 0.125, and
 10 0.0625. Purified 2H7 IgG and IgA fusion proteins were added at a concentration of 5 µg/ml. The BJAB cells, whole blood, and fusion proteins were incubated, the supernatants harvested, and the percent specific killing was calculated as described above. Percent specific killing for each of the 2H7 fusion proteins is presented in Figure 38.

The ADCC activity of purified 2H7 scFv IgG MTH (SSS) WTCH2CH3
 15 (5 µg/ml) and of purified 2H7 scFv IgA IgACH2CH3 (5 µg/ml) was compared in the presence of different effector cell populations. PBMC were isolated from whole blood as described in Examples 11 and 12. PBMC were combined with labeled BJAB target cells (5 x 10⁴ per well of 96 well tissue culture plate) at ratios of 50:1, 25:1, and 12.5:1. The assay was performed and the data analyzed as described above. Figure 39A shows that
 20 only the 2H7 scFv IgG MTH (SSS) WTCH2CH3 fusion protein had ADCC activity when PBMC served as the effector cells. Figure 39B shows that both 2H7 scFv IgG MTH (SSS) WTCH2CH3 and 2H7 scFv IgA IgACH2CH3 exhibit ADCC activity when whole blood was the source of effector cells (as illustrated in Figure 38).

EXAMPLE 20

EXPRESSION LEVEL OF 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 FUSION 25 PROTEIN

This Example compares the expression level of 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO:___) with other 2H7 scFv IgG constructs that do not contain the mutation in the variable heavy chain domain. The
 30 mammalian expression vector pD18 comprising nucleotide sequences 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:); 2H7 scFv IgG WTH

(CCC) WTCH2CH3 (SEQ ID NO:); and 2H7 scFv VHSE11 IgG MTH (SSS) WTCH2CH3 (see Examples 1 and 13) were transiently transfected into COS cells as described in Example 10. After 72 hours at 37 °C, culture supernatants were harvested, and 1 µl of each supernatant was combined with non-reducing sample buffer (see method described in Example 10). The culture supernatant samples and aliquots of purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 (40 ng, 20 ng, 10 ng/ 5 ng, and 2.5 ng) were fractionated on 10% Bis-Tris (MOPS) NuPAGE® gels (Invitrogen Life Technologies). Multimer® protein standards (Invitrogen Life Technologies) were also separated on the gel. The proteins were transferred to a PDVF membrane and immunoblotted as described in Example 17. The immunoblot is presented in Figure 40. The amounts of the fusion proteins were quantified by densitometry analysis of the blots using the ScionImage for Windows software and comparison with the standard curve. The 2H7 scFv IgG WTH (CCC) WTCH2CH3 construct produced approximately 12 ng/ul or 12 micrograms/ml, the 2H7 scFv IgG MTH (CSS) WTCH2CH3 produced approximately 10 ng/ul or 10 micrograms/ml, the 2H7 scFv IgG MTH (SCS) WTCH2CH3 construct produced approximately 1 ng/ul or 1 microgram/ml, and the 2H7 scFv VHSE11 IgG MTH (SSS) WTCH2CH3 construct produced approximately 30 ng/ml or 30 micrograms/ml. In particular claimed embodiments the instant invention, an amino acid sequence of 2H7 scFv VHSE11 IgG MTH (SSS) WTCH2CH3, or a polynucleotide sequence that encodes 2H7 scFv VHSE11 IgG MTH (SSS) WTCH2CH3 may be optionally excluded from the instant invention. Similarly, an amino acid sequence of 2H7 scFv VHSE11 IgG WTH (CCC) WTCH2CH3, or a polynucleotide sequence that encodes 2H7 scFv VHSE11 IgG WTH (CCC) WTCH2CH3 may be optionally excluded from particular claimed embodiments of the instant invention. Additionally, an amino acid substitution of a leucine at position 11 to serine in the variable heavy chain domain, or polynucleotides that encode an amino acid substitution of a leucine at position 11 to serine in the variable heavy chain domain, may be optionally excluded from particular claimed embodiments of the instant invention.

EXAMPLE 21

CONSTRUCTION OF A 2H7 scFv IgG FUSION PROTEIN WITH A MUTANT CH3 DOMAIN

Amino acid mutations were introduced into the CH3 domain of a 2H7 IgG fusion protein. The pD18 vector comprising 2H7 scFv IgG MTH (SSS) WTCH2CH3

(SEQ ID NO:__) was digested with BclI and XbaI to remove the MTH WTCH2CH3 (SEQ ID NO:__) fragment, which was then subcloned into pShuttle vector (BD Biosciences Clontech, Palo Alto, CA) that was double-digested with BclI and XbaI. Subcloning was performed in a kanamycin resistant vector because the ampicillin resistance gene has an XmnI site, which is required for this cloning procedure. Five constructs were prepared with the following substitutions: (1) a phenylalanine residue at position 405 (numbering according to Kabat *et al. supra*) was substituted with tyrosine using the oligonucleotide CH3Y405; (2) the phenylalanine position at 405 was substituted with an alanine residue using the oligonucleotide CH3A405; (3) the tyrosine residue at position 407 was substituted with an alanine using the oligonucleotide CH3A407; (4) both wild type amino acids at positions 405 and 407 were substituted with tyrosine and alanine, respectively using the oligonucleotide CH3Y405A407; and (5) both wild type amino acids at positions 405 and 407 were substituted with alanine using the oligonucleotide CH3A405A407. The oligonucleotides were the 3' primers for PCR amplification of a portion of the CH3 domain. The nucleotide sequences for each 3' oligonucleotide were as follows.

CH3Y405: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct gta gag gta gaa gga gcc-3' (SEQ ID NO:__)

CH3A405: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct gta gag ggc gaa gga gcc-3' (SEQ ID NO:__)

CH3A407: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag gaa gaa gga gcc-3' (SEQ ID NO:__)

CH3Y405A407: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag gta gaa gga gcc-3' (SEQ ID NO:__)

CH3A405A407: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag ggc gaa gga gcc-3' (SEQ ID NO:__)

The template was the mutant hinge MHWTC2CH3 human IgG1. The 5' PCR oligonucleotide primer was huIgGMHWC, [SEQ ID NO:__]. The amplified products were TOPO® cloned and sequenced as described in Examples 1 and 10. DNA from the clones with the correct sequence was digested with BclI and XmnI and transferred to pShuttle containing the MTH WTCH2CH3 sequence, which was also digested with the

same restriction enzymes. The mutated IgG sequences were then removed by digestion with BclI and XbaI and inserted into a pD18 vector containing 2H7 scFv that was also digested with BclI and XbaI. The polynucleotide sequences for mutated the CH3 domains, MTCH3 Y405, MTCH3 A405, MTCH3 A407, MTCH3 Y405A407, and MTCH3 A405A407 are shown in SEQ ID NOs: ____, respectively, and the polypeptide sequences for each are shown in SEQ ID NOs: ____, respectively. The polynucleotide sequences for the 2H7 scFv MTH WTCH2 MTCH3 Y405, 2H7 scFv MTH WTCH2 MTCH3 A405, scFv MTH WTCH2 MTCH3 A407, scFv MTH WTCH2 MTCH3 Y405A407, and scFv MTH WTCH2 MTCH3 A405A407, respectively, and the deduced amino acid sequences are shown in SEQ ID NOs: ____, respectively.

EXAMPLE 22

CONSTRUCTION OF 2H7 scFv IgG FUSION PROTEINS WITH HINGE MUTATIONS

A 2H7 scFv IgG fusion protein was constructed with the third cysteine residue in the IgG1 hinge region substituted with a serine residue. The template for introduction of the mutations was a polynucleotide encoding 2H7 scFv WTH WTCH2CH3 (SEQ ID NO: ____). The oligonucleotide introducing the mutations was a 5' PCR primer oligonucleotide HIgGMHcys3 having the sequence 5'-gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tcc cca gca cct-3'. The oligonucleotide introducing the mutation into the hinge region was combined with template and a 3' oligonucleotide containing an XbaI site (underlined and italicized) (5'-gtt gtt *tct aga* tca tt acc cgg aga cag gga gag gct ctt ctg cgt gta g-3' (SEQ ID NO: ____)) to amplify the mutant hinge-wild type (WT)-CH2-CH3 sequences by PCR. The IgG MTH CCS mutant sequence was amplified for 30 cycles with a denaturation profile of 94 °C, annealing at 50 °C for 30 seconds, and extension at 72 °C for 30 seconds. The amplified polynucleotides were inserted into the TOPO® cloning vector (Invitrogen Life Technologies) and then were sequenced as described in Example 1 to confirm the presence of the mutation. pD18 vector containing 2H7 scFv was digested to remove the constant region sequences essentially as described in Example 10. The mutant hinge-wild type CH2-CH3 regions were inserted in frame into the digested vector DNA to obtain vectors comprising 2H7 scFv MTH (CCS) WTCH2CH3 encoding DNA (SEQ ID NO: ____). The deduced polypeptide sequence is shown in SEQ ID NO: ____.

EXAMPLE 23

CONSTRUCTION OF ANTI-CD20 IGE FUSION PROTEINS

A binding domain is fused to IgE constant region sequences such that the expressed polypeptide is capable of inducing an allergic response mechanism. The single chain Fv nucleotide sequence of 40.2.220 (SEQ ID NO:__), an anti-CD40 antibody, is fused to IgE CH2-CH3-CH4 according to methods described for other scFv immunoglobulin constant region constructs (see Examples 1, 5, 10, and 13). To PCR amplify the IgE CH2-CH3-CH4 domains, a 5' oligonucleotide primer, hIgE5Bcl, having the sequence 5'-gtt gtt gat cac gtc tgc tcc agg gac ttc acc cc-3', and a 3' oligonucleotide primer, hIgE3stop, having the sequence 5'- gtt gtt tct aga tta act ttt acc ggg att tac aga cac cgc tcg ctg g-3' are used.

The retroviral transfection system for ectopic surface expression of genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors described in Example 12 is used to construct a 40.2.220 scFv IgE-CD80 fusion protein. The 40.2.220 scFv IgE fusion polynucleotide sequence is fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO:__), such that when the fusion protein is expressed in the transfected cell, CD80 provided an anchor for surface expression of the scFv Ig fusion protein. cDNA encoding the anti-CD40 scFv-IgE-CD80 fusion proteins (SEQ ID NO:__) is inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions. The 40.2.220 scFv-Ig-CD80 cDNA is inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence. The retroviral constructs are transfected into a carcinoma cell line, and transfected cells are screened to select clones that are expressing the 40.2.220 scFv-Ig-CD80 fusion protein on the cell surface.

EXAMPLE 24

CONSTRUCTION OF IGA-T4 MUTANTS THAT ARE EXPRESSED ON THE CELL SURFACE

The retroviral transfection system for ectopic surface expression of genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors described in Example 12 is used to construct a 2H7 scFv IgA hinge IgA-T4-CD80 fusion protein. The 2H7 scFv IgAH IgA-T4 fusion polynucleotide sequence (SEQ ID NO:__) is fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO:__), such that when the fusion protein is

expressed in the transfected cell, CD80 provided an anchor for surface expression of the scFv Ig fusion protein. cDNA encoding the 2H7 scFv IgAH IgA-T4-CD80 fusion protein (SEQ ID NO:__) is inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions. The 2H7
5 scFv IgAH IgA-T4-CD80 cDNA is inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence. The retroviral construct is transfected into Reh, an acute lymphocytic leukemia cell line (ATCC CRL-8286). Transfected cells are screened to select clones that are expressing 2H7 scFv-Ig fusion proteins on the cell surface.

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EXAMPLE 25

CHARACTERIZATION OF AN ANTI-4-1BB scFv Ig-CD80 FUSION PROTEIN EXPRESSED ON THE CELL SURFACE OF TUMOR CELLS AND GROWTH OF THE TUMOR CELLS *IN VIVO*

This Example describes construction of an anti-murine 4-1BB (CD137) scFv fusion protein that has an IgG wild type hinge and CH2 and CH3 domains that is
15 fused to the CD80 transmembrane and cytoplasmic domains. The Example also illustrates the effect of the cell surface expression of the anti-4-1BB scFv IgG CD80 polypeptide when the transfected tumor cells are transplanted into mice.

The heavy and light chain variable regions of a rat anti-4-1BB (CD137) monoclonal antibody (1D8) were cloned, and a single chain Fv construct was prepared
20 essentially as described in Example 1. The heavy chain and light chain variable regions of each antibody were cloned according to standard methods for cloning immunoglobulin genes and as described in Example 1. A single chain Fv construct was prepared as described in Example 1 by inserting a nucleotide sequence encoding a (gly₄ser)₃ peptide linker between the VL region nucleotide sequence of 1D8 (SEQ ID NO:__) and the VH region
25 nucleotide sequence of 1D8 (SEQ ID NO:__). The polypeptide sequence for 1D8 VL is shown in SEQ ID NO:__, and the polypeptide sequence for the VH domain is shown in SEQ ID NO:__. The scFv polynucleotide (SEQ ID NO:__) was then fused to human IgG1 wild-type hinge-CH2-CH3 domains according to the methods described in Example 1. The scFv IgG1 fusion polynucleotide sequence was then fused in frame to sequences
30 encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO:__) essentially as described in Example 12, such that when the fusion protein was expressed in the transfected cell, CD80 provided an anchor for surface expression of the

scFv Ig fusion protein. cDNA encoding the scFv-IgG-CD80 fusion protein (SEQ ID NO:___) was inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions. The scFv-Ig-CD80 cDNA was inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence.

The retroviral constructs were transfected into the metastatic M2 clone of K1735, a melanoma cell line, provided by Dr. I. Hellstrom, PNRI, Seattle, WA. Transfected cells were screened to select clones that were expressing scFv-Ig fusion proteins on the cell surface. To demonstrate that the 1D8 scFv IgG-CD80 construct was expressed on the cell surface of the tumor cells, the transfected cells were analyzed by flow immunocytofluorimetry. Transfected cells (K1735-1D8) were incubated for one hour on ice in phycoerythrin-conjugated F(ab')₂ goat anti-human IgG. The unbound conjugate was then removed by washing the cells and flow cytometry analysis was performed using a Coulter Epics XL cell sorter. Results are presented in Figure 41A.

The growth of K1735-1D8 transfected cells was examined *in vivo*. K1735-WT cells grew progressively when transplanted subcutaneously (s.c.) in naïve C3H mice. Although the same dose of K1735-1D8 cells initially formed tumors of an approximately 30 mm² surface area, the tumors started to regress around day 7 and had disappeared by day 20 as shown in Figure 41B. Tumor cells that were transfected with a similarly constructed vector encoding a non-binding scFv, a human anti-CD28 scFv construct, grew as well as tumor cells that had not been transfected. The presence of a foreign protein, that is, human IgG1 constant domains or rat variable regions, did not make transfected K1735-WT cells immunogenic; the growth of the K1735-1D8 cells in C3H mice was identical to that of K1735-WT cells (untransfected).

To investigate the roles of CD4⁺ and CD8⁺ T lymphocytes and NK cells in the regression of K1735-1D8 tumors, naïve mice were injected intraperitoneally (i.p.) with monoclonal antibodies (monoclonal antibodies, typically 50 µg in a volume 0.1 ml) to remove CD8⁺, CD4⁺ or both CD4⁺ and CD8⁺ T cells, or were injected with anti-asialo-GM1 rabbit antibodies to remove NK cells. Twelve days later, when flow cytometry analysis of spleen cells from identically treated mice showed that the targeted T cell populations were depleted, K1735-1D8 cells were transplanted s.c to each T cell-depleted

group. K1735-1D8 had similar growth kinetics in mice that had been injected with the anti-CD8 MAb or control rat IgG while removal of CD4⁺ T cells resulted in the growth of K1735-1D8 with the same kinetics as K1735-WT. This failure to inhibit tumor growth after CD4⁺ T cell removal was observed regardless of the presence or absence of CD8⁺ T cells. K1735-1D8 grew in all NK-depleted mice, although more slowly than in the CD4-depleted group. The results are presented in Figure 41C.

EXAMPLE 26

THERAPEUTIC EFFECT OF TUMOR CELLS EXPRESSING ANTI-4-1BB scFv IgG-CD80

FUSION PROTEIN

This Example examines the ability of K1735-1D8 transfected tumor cells expressing an anti-CD137 scFv on the cell surface to generate a sufficient immune response in mice to mediate rejection of established, untransfected wild type tumors. C3H mice were transplanted with K1735-WT tumors (2x10⁶ cells/animal) and grown for approximately six days. Experiments were performed using mice with established K1735-WT tumors of 30 mm² surface area. Mice were vaccinated by s.c. injection of K1735-1D8 or irradiated K1735-WT cells on the contralateral side. Identical injections were repeated at the time points indicated in Figure 42. One group of animals was given four weekly injections of K1735-1D8 cells. According to the same schedule, another group was given irradiated (12,000 rads) K1735-WT cells, and a third group was injected with PBS. The data are plotted in Figure 42. The WT tumors grew progressively in all control mice and in all mice that received irradiated K1735-WT cells. In contrast, the tumors regressed in 4 of the 5 mice treated by immunization with K1735-1D8. The animals remained tumor-free and without signs of toxicity when the experiment was terminated 3 months later. In the fifth mouse, the tumor nodule decreased in size as long as the mouse received K1735-1D8 cells, but the tumor grew back after therapy was terminated.

In another experiment with 5 mice/group, mice were injected intravenously (i.v.) with 3 x 10⁵ K1735-WT cells to initiate lung metastases. Three days later, K1735-1D8 cells were transplanted s.c. This procedure was repeated once weekly for a month; control mice were injected with PBS. The experiment was terminated when one mouse in the control group died, 37 days after receiving the K1735-WT cells. At that time, lungs of the control mice each had >500 metastatic foci. In contrast, less than 10 metastatic foci were present in the lungs of the immunized mice.

In a third experiment, mixtures of K1735-WT cells and K1735-1D8 cells were injected into immunocompetent syngeneic C3H mice. Mice were injected subcutaneously with K7135-WT cells alone or with a mixture of 2×10^6 K1735-WT cells and 2×10^5 K1735-1D8 cells. Tumor growth was monitored at 5-day intervals.

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EXAMPLE 27

EXPRESSION OF ANTI-4-1BB scFv IgG-CD80 FUSION PROTEIN ON THE CELL SURFACE OF SARCOMA CELLS

This Example demonstrates expression of an anti-CD137 scFv on the cell surface of a second type of tumor cell by transfecting a murine sarcoma cell line with an anti-CD137 scFv IgG-CD80 construct.

The 1D8 scFv IgG WTH WTCH2CH3-CD80 polynucleotide (SEQ ID NO:__) was transferred from the pLNCX vector into pCDNA3-hygro vector using restriction enzyme digestion and ligation steps according to standard molecular biology methods. The construct was cut with HindIII + ClaI and the scFv fragment was filled in with Klenow (Roche) and the blunt-ended fragment was ligated into EcoR5 site of pCDNA3. Ag104 murine sarcoma tumor cells were transfected with the pCDNA3-hygro vector containing the 1D8 scFv IgG CD80 fusion protein. Hygromycin-resistant clones were screened by flow cytometry using a FITC anti-human IgG antibody to detect expression of the transgene. Only approximately 15% of the resistant clones had detectable fusion protein initially. Positive cells identified by flow cytometry were repeatedly panned on flasks coated with immobilized anti-human IgG (10 μ g/ml) according to standard methods. Panning was performed by incubating cells on the coated plates for 30 min at 37°C; the plates were then washed 2-3x in versene or PBS. After each round, cells were tested for IgG expression by FACS. The histogram in Figure 44 shows the staining pattern after four rounds of panning against anti-human IgG (black). Untransfected cells were stained and are indicated in gray. All of the cells in the population were positive.

EXAMPLE 28

CONSTRUCTION AND CHARACTERIZATION OF A BISPECIFIC scFv Ig FUSION PROTEIN AND scFv Ig FUSION PROTEINS WITH A MUTATION IN THE IGG1 CH2 DOMAIN

An anti-CD20 (2H7) scFv IgG fusion protein was constructed that had a mutant hinge (MT (SSS)) and a mutant CH2 domain in which the proline at residue

(position number 238 according to Ward *et al.*, *supra*) was substituted with a serine. The 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 encoding polynucleotide (SEQ ID NO:___) was constructed essentially according to methods described in Examples 1, 5, and 13. The IgG mutant hinge-mutant CH2-wild type CH3 domains were also fused to an anti-CD20 (2H7)-anti-CD40 (40.2.220) bispecific scFv. The anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3 encoding polynucleotide sequence is shown in SEQ ID NO:___ and the encoded polypeptide is shown in SEQ ID NO:___.

COS cells were transiently transfected with vectors comprising the polynucleotide sequences encoding 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (SEQ ID NO:___); anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3 (SEQ ID NO:___); 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:___); and 2H7 scFv IgAH IgG WTCH2CH3 (SEQ ID NO:___) as described in Example 10. Culture supernatants were collected and the fusion proteins were purified by protein A chromatography (see Example 10). The purified polypeptides were fractionated by SDS-PAGE according to the method described in Example 10. Rituximab (anti-CD20 monoclonal antibody), and Bio-Rad prestained molecular weight standards (Bio-Rad, Hercules, CA), and Multimark® molecular weight standards (Invitrogen Life Technologies) were also applied to the gel. The results are presented in Figure 45.

The 2H7 scFv Ig fusion protein that contains a mutation in the CH2 domain was compared to fusion proteins that have the wild type CH2 domain in an ADCC assay. The assays were performed essentially as described in Examples 11 and 19. Fresh resting PBMC (effector cells) were added to ⁵¹Cr-labeled BJAB cells (target cells) at the ratios indicated in Figure 46. Purified 2H7 scFv IgG MTH (SSS) MTCH2WTCH3, 2H7 scFv IgG MTH (SSS) WTCH2CH3, 2H7 scFv IgAH IgG WTCH2CH3, and Rituximab, each at 10 µg/ml were added to the effector/target cell mixtures and incubated for five hours at 37 °C. Supernatants were harvested and the amount of chromium released was determined as described in Examples 11 and 19. Percent specific killing by each fusion protein is presented in Figure 46.

EXAMPLE 29

TUMOR CELL SURFACE EXPRESSION OF AN ANTI-HUMAN CD3 SCFV IGG FUSION

PROTEIN

An anti-human CD3 scFv Ig CD80 fusion protein was prepared essentially as described in Examples 1 and 12. The fusion protein comprised an anti-human CD3 scFv fused to wild type IgG1 hinge (SEQ ID NO:___) and wild type CH2 (SEQ ID NO:___) and CH3 (SEQ ID NO:___) domains, fused to CD80 transmembrane and cytoplasmic domains (SEQ ID NO:___) to enable cell surface expression of the anti-CD3 scFv. The anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 polynucleotide (SEQ ID NO:___) encoding the polypeptide (SEQ ID NO:___) was transfected in Reh cells and into T51 cells (lymphoblastoid cell line). Expression of the anti-human CD3 scFv IgG fusion protein was detected by flow cytometry using FITC conjugated goat anti-human IgG (see methods in Examples 4, 10, 16, 18). Figure 47A illustrates expression of the anti-human CD3 fusion protein on the cell surface of Reh cells, and figure 47B shows expression of the fusion protein on T41 cells.

ADCC assays were performed with the transfected Reh and T51 cells to determine if expression of the scFv-Ig polypeptides on the cell surface augmented effector cell function. Untransfected and transfected Reh cells and untransfected and transfected T51 cells were pre-labeled with ^{51}Cr (100 μCi) (Amersham) for two hours at 37 °C. Human PBMC served as effector cells and were added to the target cells (5×10^4 cells per well of 96 well plate) at ratios of 20:1, 10:1, 5:1, and 2.5:1. After four hours at 37 °C, culture supernatants were harvested and analyzed as described in Examples 11 and 12. Percent specific killing was calculated as described in Example 12. The results are presented in Figure 48.

EXAMPLE 30

INDUCTION OF CYTOKINE EXPRESSION IN TUMOR CELLS EXPRESSING

ANTI-CD28 scFv ON THE CELL SURFACE

This Example describes the effect of cell surface expressed scFv on cytokine mRNA induction in stimulated lymphocytes co-cultured with tumor cells transfected with an anti-human CD28 scFv IgG-CD80 fusion protein.

Real time PCR analysis was performed on RNA samples from human PBMC stimulated with Reh, Reh-anti-CD28 (2e12) (see Example 12 for construction of 2e12 scFv IgG WTH WHTCH3CH2-CD80 and transfection of Reh cells), and Reh-CD80 (see Example 14) in order to measure the effects of the surface expressed scFv on cytokine production by the PBMC effector cells. For the real-time PCR assay, SYBR Green

(QIAGEN) (Morrison *et al.*, *Biotechniques* 24:954-8, 960, 962 (1998)) was used and measured by an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) that measures the formation of PCR product after each amplification cycle. Cells were harvested from cultures and total RNA prepared using QIAGEN RNA kits, including a QIA shredder column purification system to homogenize cell lysates, and RNeasy® mini-columns for purification of RNA. cDNA was reverse transcribed using equal amounts of RNA from each cell type and Superscript II Reverse Transcriptase (Life Technologies). SYBR Green real-time PCR analysis was then performed using the prepared cDNA as template and primer pairs specific for cytokine gene products. The average length of the PCR products that were amplified ranged from 150-250 base pairs. The cDNA levels for many activation response molecules including IFN γ , TNF α , GM-CSF, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, ICOSL, CD80 and CD86 were assayed. Control reference cDNAs for constitutively expressed genes, including GAPDH, β -actin, and CD3 ϵ were measured in each assay. The most significant induction of specific mRNA was observed for IFN- γ , and more modest induction was observed for CTLA-4 and ICOS.

EXAMPLE 31

CLONING OF AN ANTI-HUMAN 4-1BB ANTIBODY AND CONSTRUCTION OF AN ANTI-HUMAN 4-1BB SCFV IG FUSION PROTEIN

A hybridoma cell line expressing a mouse anti-human monoclonal antibody (designated 5B9) was obtained from Dr. Robert Mittler, Emory University Vaccine Center, Atlanta, GA. The variable heavy and light chain regions were cloned according to known methods for cloning of immunoglobulin genes and as described herein. Cells were grown in IMDM/15% FBS (Invitrogen Life Technologies) media for several days. Cells in logarithmic growth were harvested from cultures and total RNA prepared using QIAGEN RNA kits, including a QIA shredder column purification system to homogenize cell lysates, and RNeasy® mini-columns for purification of RNA according to manufacturer's instructions. cDNA was reverse transcribed using random hexamer primers and Superscript II Reverse Transcriptase (Invitrogen Life Technologies).

cDNA was anchor-tailed using terminal transferase and dGTP. PCR was then performed using an anchor-tail complementary primer and a primer that annealed specifically to the antisense strand of the constant region of either mouse C κ (for

amplification of VL) or the appropriate isotype mouse CH1 (for amplification of VH). The amplified variable region fragments were TOPO® cloned (Invitrogen Life Technologies), and clones with inserts of the correct size were then sequenced. Consensus sequence for each variable domain was determined from sequence of at least four independent clones.

5 The 5B9 VL and VH polynucleotide sequences are shown in SEQ ID NOs:___ and ___, respectively, and the deduced amino acid sequences are shown in SEQ ID NOs:___ and _____. The scFv was constructed by a sewing PCR method using overlapping primers containing a synthetic (Gly₄Ser)₃ linker domain inserted between the light and heavy chain variable regions (see Example 1). The 5B9 scFv polypeptide (SEQ ID NO:___) is encoded by the
10 polynucleotide sequence comprising SEQ ID NO:_____.

5B9 scFv polynucleotide sequence was fused in frame to the polynucleotide sequence encoding the human IgG1 mutant hinge and wild type CH2 and CH3 (MTH (SSS) WTCH2CH3, SEQ ID NO:___) according to methods described in Examples 5, 10, and 13. COS cells were transiently transfected with a vector comprising the 5B9 scFv IgG
15 MTH (SSS) WTCH2CH3 polynucleotide sequence (SEQ ID NO:___). Supernatant was collected and binding of the 5B9 scFv IgG MTH (SSS) WTCH2CH3 polypeptide (SEQ ID NO:___) was measured by flow immunocytofluorimetry essentially as described in Examples 4, 10, 16, and 18. Culture supernatant from the 5B9 hybridoma cell line was also included in the binding assay. Fresh human PBMC were incubated in the presence of
20 immobilized anti-CD3 for four days prior to the binding experiment to induce expression of CD137 on the surface of activated T cells. Stimulated PBMC were washed and incubated with COS or hybridoma culture supernatant containing the 5B9 scFv IgG fusion protein or 5B9 murine monoclonal antibody, respectively, for 1 hour on ice. Binding of 5B9 scFv IgG fusion protein or 5B9 murine monoclonal antibody was detected with FITC
25 conjugated anti-human IgG or anti-mouse IgG, respectively. The results are presented in Figure 49.

EXAMPLE 32

CONSTRUCTION OF 2H7 scFv IgG FUSION PROTEINS WITH HINGE MUTATIONS

2H7 scFv IgG fusion proteins are constructed with the first cysteine residue
30 and the second cysteine in the IgG1 hinge region substituted with a serine residue to provide MTH (SCC) and MTH (CSC). The template for introduction of the mutations is a polynucleotide encoding 2H7 scFv WTH WTCH2CH3 (SEQ ID NO:___). The

oligonucleotide introducing the mutations are 5' PCR primer oligonucleotides HIgGMHcys1 (SEQ ID NO:__) and HIgGMHcys2 (SEQ ID NO:__). The constructs are prepared as described in SEQ ID NO:__). The encoding polynucleotides of the mutants are presented in SEQ ID NOs:__) and the polypeptide sequences are provided in SEQ ID NO:__).

Example 33

CONSTRUCTION OF 2H7 V_HL11S scFv (SSS-S) H WCH2 WCH3

A change from leucine to serine at position 11 in the heavy chain variable region (numbering according to Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)) was introduced into the 2H7 scFv MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO:__). The wild type leucine residue was substituted with serine by site-directed mutagenesis using the oligonucleotide V_Hser11: 5'-gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc-3' (SEQ ID NO:__). The 3'-primer for PCR was huIgG1-3' having the sequence 5'-gtc tct aga cta tca ttt acc cgg aga cag-3' (SEQ ID NO:__) (XbaI site underlined and italicized). After PCR amplification, the fragments were inserted into the TOPO® cloning vector and sequenced to confirm the presence of the V_H11 leucine to serine mutation. The 2H7 scFv-IgG (SSS-S) H WCH2 WCH3 encoding DNA was shuttled into the PSL1180 cloning vector (Pharmacia Biotech, Inc., Piscataway, NJ). The construct PSL1180-2H7 scFv-IgG (SSS-S) H WCH2 WCH3 was digested with Sac and XbaI to remove the wild type V_H domain and the connecting region and CH2 and CH3 domains. The PCR product comprising the V_H11 mutant was digested with Sac and XbaI and then inserted into the digested PSL1180 construct according to standard molecular biology procedures. The construct was then digested with Hind III and XbaI, and inserted into the mammalian expression vector pD18 (see methods described in Example 1 and Example 10). The mutant is designated 2H7 scFv V_H L11S IgG (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 34

EXPRESSION AND OF 2H7 scFv V_H L11S (SSS-S) H WCH2 WCH3

IN STABLE CHO LINES

CHO DG44 cells were transfected by electroporation with approximately 150 micrograms of linearized expression plasmid encoding the 2H7 VH L11S scFv (SSS-S) H WCH2 WCH3. Cultures were plated in selection media containing 100 nM methotrexate, in 96 well, flat bottom tissue culture plates at various numbers of cells/well, ranging from 125 to 2000. Methotrexate resistant clones were selected and culture supernatants were screened for the highest expressors of the fusion protein using a CD20CHO binding assay similar to that described for Figure 1. Clones were amplified after the initial selection in gradually increasing doses of methotrexate. Cells were passaged for two passages in the higher concentration prior to adjusting the concentration to the next higher dose. Clones were amplified to a final concentration of 1 micromolar methotrexate.

Figure 50B illustrates the production levels of 2H7 VH L11S scFv (SSS-S)H WCH2 WCH3. Spent supernatants from amplified CHO cells expressing this molecule and growing in stationary T25 flasks were tested for quantitative binding to CD20 CHO cells by flow cytometry. The activity was converted to protein concentration by generation of a standard curve using the same molecule purified from supernatants with Protein A affinity chromatography (Figure 50A). The concentration of the purified protein was determined by A280 using an extinction coefficient provided by the amino acid composition of the recombinant protein (Vector NTI). Although levels of production varied between clones tested, multiple clones produced over 1 mg/ml. This level of protein expression is over 10-fold higher than the identical molecule except for the amino acid change in V_H.

Figure 51 illustrates the production levels of 2H7 VH L11S scFv (SSS-S) H WCH2 WCH3 by semi-quantitative analysis on SDS-PAGE. Ten microliters of spent supernatant from amplified CHO cells expressing this molecule and growing in stationary T25 flasks were mixed with 10 microliters 2X non-reducing SDS sample buffer, run on SDS-PAGE gels, and stained with coomassie blue.

EXAMPLE 35

CONSTRUCTION AND BINDING CAPACITY OF G28-1 scFv IG CONSTRUCTS

Contrstruction of the G28-1 (anti-CD37) scFv was performed using total RNA isolated from the G28-1 hybridoma using Trizol (Invitrogen) reagent according to manufacturer's instructions. cDNA was prepared using random primers and the protocol

described previously for 2H7 cloning in Example 1. The variable domains of the scFv was cloned using one of two methods: the first method used a family of degenerate 5' oligonucleotides specific for each V region gene family and a single 3' primer specific for the constant region of either the light or heavy chain using methods and primers described in (Ig-Prime Kit Mouse Ig-Primer Set, Novagen). The second approach used the anchor-tailing methods and primers described in (Gilliland LK et al, Tissue Antigens 47: 1-20 (1995). In either case, PCR amplified products were cloned into the TOPO cloning vector (Invitrogen). The clones were digested with EcoRI and screened for inserts of the proper size. Positive clones were sequenced as previously described in Example 1.

Specific primers were then designed for each V region, one with the leader sequence and one without. Primers were also designed to include desired linkers and/or restriction sites at the primer ends. PCR reactions were performed on the TOPO cloned DNA using a 25 cycle program with the following profile: 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, followed by a final extension at 72C for 8 minutes. PCR products were gel purified and fragments recovered using a QIAQUICK gel extraction kit (QIAGEN, Valencia, CA). Fragments were diluted 1:50 and 1 microliter used for SEWING PCR reactions according to the methods described in Example 1. The following oligonucleotides were used for the secondary PCR reactions of the V_L domain for the G28-1 scFv:

5' primers with SalI site without leader: 5'-

GTTGTTGTCGACATCCAGATGACTCAGTC

TCCA-3' (SEQ ID NO__)

5' primer with HindIII site and leader sequence: 5'-

GTCAAGCTTGCCGCCATGGTATCCA

CAGCTCAGTTCCTTGG-3' (SEQ ID NO__)

3' primer: 5'-

GCCACCCGACCCACCAACCGCCCGAGCCACCGCCACCTTTGATCTCCA

GTTCGGTG CC-3' (SEQ ID NO__)

The primers used for the V_H domain are shown below:

5' sense primer:

TCGGGCGGTGGTGGGTCGGGTGGCGGCGGATCGTCAGCGGTCCAGCTGCA

GCAGTCTGGA-3' (SEQ ID NO__)

3' antisense primer with BclI site: 5'-

TCAGTGCTGATCAGAAGAGACGGTGACTGAGGTTTCCTTG-3' (SEQ ID NO__).

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the G28-1 scFv by site-directed mutagenesis. The wild type form of the G28-1 scFv was initially constructed by sewing/overlap PCR to insert a (gly4ser)3 linker between the VL and VH domains as described above. However, no Sac I site was introduced as a part of this fusion of the variable domains, so alternative, nearby restriction sites (HaeII and PvuII) near leucine 11 were used to synthesize the VL+mutated VH domain. Primers were designed to contain one of these sites and the DNA sequence including the L to S change, followed by 12 wild type base pairs. Several attempts at this strategy failed, so an alternative strategy using the Genetailor (Invitrogen) method of site directed mutagenesis was used to introduce the desired mutation. The mutagenesis was carried out according to manufacturer's instructions. Briefly the procedure involves methylation of the plasmid DNA with DNA methylase, amplification of the DNA in a mutagenesis reaction with two overlapping primers, one of which contains the target mutations, transformation of the plasmid into wild type E.coli which digests all methylated DNA and leaves only the unmethylated, mutated amplification product. Both primers are approximately 30 nucleotides in length (not including the mutation site on the mutagenic primer, with an overlapping region at the 5' ends of 15-20 nucleotides, for efficient end joining of the mutagenesis product. The template for the mutagenesis reaction was 100 ng of a plasmid containing the wild type G28-1 scFvIg construct, and the primers used for the G28-1 VH mutagenesis are as follows:

Forward primer:

5'-GCAGCAGTCTGGACCTGAGTCGGAAAAGCCTG-3' (SEQ ID NO__)

Reverse Primer:

5'-CTCAGGTCCAGACTGCTGCAGCTGGACCGC-3' (SEQ ID NO__)

PCR reactions were performed using the 15 ng methylated template, the primers above, and the usual reaction components as previously described. A 20 cycle program with the following profile was used for amplification: 94C, 30 sec; 55C, 30 sec; 68C, 8 min, followed by a final 68C extension step for 10 minutes. PCR products were transformed into wild type bacteria, and colonies screened by sequencing. Clones with

only the desired mutation were isolated and plasmid prepared as previously described Example 33. The mutant is designated G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

5 The expression level of G28-1 fusion proteins was confirmed using Immunoblot analysis according to the methods described in Example 17. Figure 53 illustrates a large increase in protein expression in the VH L11S mutant G28-1 fusion proteins compared to the G28-1 fusion protein without the mutation.

10 The G28-1 scFv Ig fusion proteins were transiently transfected and expressed in COS cells according to methods described in Example 10. Figure 52 illustrates the capacity of the G28-1 scFv Ig fusion proteins from the COS supernatants to bind CD37. Ramos and BJAB cells both express human CD37, and were therefore used to screen the G28-1 supernatants for functional activity. Binding of G28-1 scFv (SSS-S) H WCH2 WCH3 and G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 to CD37+ Ramos cells
15 was measured by flow cytometry according to methods described in Example 2. Each point on the graph represents the mean of five replicate transfections. The graph illustrates that the G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 is able to bind CD37+ Ramos cells.

20 Addition constructs were made with different connecting regions. The pD18 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3 vector was digested with BclI and XbaI to remove the connecting region, CH2 and CH3. These were replaced with each different connecting region, CH2 and CH3 according to the methods described in Example 13. The new constructs were designated: G28-1 scFv VHL11S (CSS-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (CSC-S) H WH2 WH3 (SEQ ID NO__), G28-1
25 scFv VHL11S (CSS-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (SSC-P) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (SCS-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (CCS-P) H WH2 WH3 (SEQ ID NO__), and G28-1 scFv VHL11S (SCC-P) H WH2 WH3 (SEQ ID NO__).

30 The G28-1 scFv was also attached to an IgA connecting region, CH2, CH3 and an IgE CH2, CH3, CH4. The pD18 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3 plasmid was digested using methods above to remove the connecting region CH2, and CH3. The IgA regions were inserted using methods described in Example 13. The

construct was designated G28-1 scFv VHL11S IgAH IgACH2 T4CH3 (SEQ ID NO__). The IgE CH2 CH3 CH4 region was inserted into the digested pD18 vector above using methods described in Example 39. The construct was designated G28-1 scFv VHL11S IgECH2 CH3 CH4 (SEQ ID NO__).

5

EXAMPLE 36**Characterization of 2H7 scFv Ig Mutant Fusion Proteins**

Figure 54 illustrates the binding capacity of purified 2H7 scFv Ig constructs to CD20+ CHO cells. The proteins were transfected into stable CHO cells according to methods described in Example 2. Binding was determined using flow cytometry according to the methods described in Example 2. The graph in Figure 54 illustrates that these proteins retain binding function to CD20 with altered connecting regions. Comparative results were obtained in each of the 2H7 scFv VHL11S mutants with each type of altered connecting region (results omitted).

The ability of 2H7scFv-Ig constructs with mutated connecting regions to kill CD20 positive cells in the presence of peripheral blood mononuclear cells (PBMC) through ADCC was tested by measuring the release of ⁵¹Cr from labeled BJAB cells in a 4 hr. assay using 100:1 ratio of PBMC to BJAB cells. The results shown in Figure 55 indicate that 2H7scFv-Ig mutants can mediate antibody dependent cellular cytotoxicity (ADCC), since the release of ⁵¹Cr was significantly higher in the presence of both PBMC and 2H7scFv-Ig than in the presence of either PBMC or 2H7scFv-Ig alone. Comparative results were obtained in each of the 2H7 scFv VHL11S mutants with each type of altered connecting region (results omitted).

The ability of 2H7scFv-Ig mutant fusion proteins to kill CD20 positive cells in the presence of complement was tested using B cell lines Ramos target cells. Rabbit complement was purchased from Pel-Freez (Rogers, AK), and was used in the assay at a final concentration of 1/10. Purified 2H7scFv-Ig was incubated with B cells and complement for 45 minutes at 37°C, followed by counting of live and dead cells by trypan blue exclusion. The results in Figure 56 show that 2H7scFv-Ig mutants in the presence of rabbit complement lysed B cells expressing CD20.

30

EXAMPLE 37

COMPARATIVE BINDING OF IGA, IGG, AND IGE 2H7 scFv CONSTRUCTS

Binding capacity of Ig constructs IgA, IgG and IgE were measured using flow cytometry according to the methods described in Example 2, using a commercially available (Caltag) second step specific for each Ig tail. The results in Figure 57 show that all the IgE constructs were able to bind CD20+ CHO cells comparable to the binding abilities of IgG and IgA. These results also demonstrate that the IgE constructs were detected with the IgE second step, but not the IgA or IgG second step.

EXAMPLE 38**CONSTRUCTION AND CHARACTERIZATION OF 2H7 VH L11S IGE CONSTRUCTS**

IgE tail RNA was isolated from SKO-007 cells(ATCC) using QIAGEN QIAshredder homogenization and RNA minikits. Random-primed cDNA was generated according to the usual protocol, with 4 microliters RNA eluted from the QIAGEN columns. Human IgE from the beginning of CH1 through CH4 (approximately 1.2 kb) was isolated by PCR amplification of 5 microliters cDNA, with an amplification profile of 94C, 60 sec; 72C, 2 minutes for 35 cycles, and the following primers:

5' primer: 5'-ggatccaccgctgctgcaaaaacattccctccaatgccacctccgtgac-3' (SEQ ID NO__)

3' primer: 5'-tcatttaccgggattacagacaccgctcgctggacgggtctgtgaggggctcgctgc-3' (SEQ ID NO__)

PCR fragments were ligated into PCR 2.1-TOPO vector, and transformants screened for inserts of the correct size by digestion with EcoRI according to the methods described in Example 1. One of the clones with the correct sequence from was used as template to amplify the CH2-CH4 domains with appropriate restriction sites attached for subcloning as soluble or cell surface (ORF) forms. The following primers were used with an amplification profile of 94C, 60 sec; 55C, 60 sec; 72C, 2 min; for 35 cycles to amplify a fragment of approximately 950 bp:

5' primer: (attaches BclI site to 5' end of CH2 domain of IgE)

5'-gttggtgatcacgtctgctccagggacttcacc-3' (SEQ ID NO__)

3' primer: (attaches stop codon and XbaI site to 3' end of CH4 of IgE)

5'-gttggttctagattatcatttaccaggattacagacaccgctcgctg-3' (SEQ ID NO__)

3' primer: (attaches SfuI and BamHI to 3' end of CH4 without a stop codon)

5'-gttggtttcgaaggatccgctttaccagatttacagacaccgctcgctg-3' (SEQ ID NO__)

The IgE CH2CH3CH4 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 IgECH2CH3CH4. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__. The IgE CH2CH3CH4 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted in into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 IgECH2CH3CH4(ORF). The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

The human IgE was also amplified as a fragment missing both CH1 and CH2 domains, with only the CH3 and CH4 domains attached to the human IgG1 hinge. Sequential PCR reactions using overlapping 5' oligonucleotides were used to attach the IgG1 hinge to the CH3 domain of human IgE. Primers for the first step of the PCR reaction:

5' Primer: 5'-actcacacatccccaccgtccccagcatccaacccgagaggggtgagc-3' (SEQ ID NO__)

Primers for the second step of the PCR reaction:

5' primer: 5'-tctgatcaggagcccaaatcttctgacaaaactcacacatccccaccg-3' (SEQ ID NO__)

3' primer: 5'-gtgtttctagattatcattaccaggatttacagacaccgctcgctg-3' (SEQ ID NO__)

The PCR product was digested with EcoRI and sequenced according to the methods described in Example 1. Positive clones were inserted into pD18 plasmid containing 2H7 VHL11S scFv (SSS-S) H. The construct was designated 2H7 VHL11S scFv (SSS-S) H IgE CH3CH4. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

Binding capacity of 2H7 scFv VH L11S IgECH2 CH3 CH4, was measured using flow cytometry, essentially according to Example 2. The protein was purified using MEP HyperCel, (Cipergen, Catalog # 12035-010, Lot# 200920/0271) chromatography resin and Hydrophobic Charge Induction Chromatography (HCIC). HCIC absorbent is a high capacity, high selectivity, absorbent designed for capture and purification of monoclonal and polyclonal antibodies from various sources including cell culture supernatants. Columns were packed with a 10 ml bead volume of MEP Hypercel, and equilibrated with PBS, pH 7.4 containing 0.1% NaN3. Approximately 1 liter of 2H7 scFv VHL11S IgE CH2CH3CH4 CHO culture supernatant was then run over the column. A series of citrate buffers ranging from pH 3-6 were prepared for elution of the fusion

protein. The column was washed in PBS. Protein was eluted in fifteen 1 ml fractions at pH6, 5, and 4. A final 15 ml fraction was collected at pH 3.5. Aliquots from each fraction were analyzed for A280 and were also subjected to SDS-PAGE, loading roughly 10 micrograms/well based on the A280 reading. The results of these two analyses indicated that the bulk of the protein did not elute in citrate buffers at the higher pH, but eluted at pH4, and the post elution wash at pH3.5 also contained significant amounts of protein.

The ability of these 2H7 VH L11S IgE purified proteins to bind CD20+ CHO cells was determined using flow cytometry according to the methods described in Example 2 using FITC-conjugated goat-anti-human IgE. Figure 58A illustrates that both purified proteins are able to bind CD20+ CHO cells.

The ability of these 2H7 VH L11S IgE purified proteins to mediate ADCC against BJAB target cells with PBMC effectors was measured according to the methods described in Example 2. Figure 58B illustrates that both proteins were able to mediate ADCC at similar levels.

EXAMPLE 39

CONSTRUCTION AND BINDING CAPACITY OF scFv VH L11S MUTANTS WITH MOUSE IGA AND IGE TAIL REGIONS

Murine IgA was cloned from murine spleen RNA using essentially the same methods used to clone the human IgE tails in Example 38. The PCR reactions were performed with a 94C 60 sec; 52C 60 sec; 72C 2 min amplification profile for 35 cycles. The PCR primers used to clone CH1-CH4 regions were:

5' primer: 5'-atctgttctcctctactactctcctccacct-3' (SEQ ID NO __)

3' primer: 5'-tcagtagcagatgccatctccctctgacatgatgacagacacgct-3' (SEQ ID NO __)

PCR primers used to delete the CH1 region:

5' primer: 5'-gttggtgatcacatctgttctcctctactactcctcctccacct-3' (SEQ ID NO __)

3' primer with a stop codon, XbaI site at end of Ig tail, and the T4 mutation in CH3 region:

5'-gttggttctagattatcaatctccctctgacatgatgacagacac-3' (SEQ ID NO __)

3' primer for the ORF, a SfuI and BamHI sites, and T4 mutation in the CH3 region:

5'-gttcttcgaaggatccgcatctccctctgacatgatgac-3' (SEQ ID NO __)

The mouse IgACH2 T4CH3 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv and the IgAH. This construct was designated 2H7 VHL11S scFv IgAH mIgACH2 T4CH3. The

polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

The mouse IgACH2 T4CH3 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted in into a pD18 vector that contains 2H7 VHL11S scFv and IgAH. This construct was designated 2H7 VHL11S scFv IgAH mIgACH2 T4CH3 (ORF). The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

Murine IgE was cloned murine IgE La2 (ATCC) RNA essentially according the methods described in Example 38. The PCR reactions were performed with a 94C 60 sec; 52C 60 sec; 72C 2 min amplification profile for 35 cycles. Initial PCR primers used to clone the CH1-CH4:

5' primer: 5'-tctatcaggaaccctcagctctacccttgaagccctg-3' (SEQ ID NO__)

3' primer: 5'-gttggttctagattatcaggatggacggaggagggtgttaccaggct-3' (SEQ ID NO__)

PCR primers to remove the CH1 region:

5' primer: 5'-gttggtgatcaggttcgacgtgtcaacatcactgagcccacc-3' (SEQ ID NO__)

3' primer with stop codon and XbaI site:

5'-gttggttctagattatcaggatggacggaggagggtgttaccaggct-3' (SEQ ID NO__)

3' primer ORF, SfuI and Bam HI: 5'-gttggtttcgaaggatccgcggatggacggaggagggtgtta-3' (SEQ ID NO__)

The mouse IgE CH2CH3CH4 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 VHL11S mIgECH2CH3CH4. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

The mouse IgE CH2CH3CH4 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted in into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 VHL11S scFv mIgECH2CH3CH4(ORF). The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

Binding capacity of 2H7 VH L11S mIgE and mIgA (with mouse tail regions) to CD20+ CHO cells were also measured by flow cytometry according to the methods described in Example 2 using commercially available IgE or IgA second step

reagents (Caltag). Each point in Figure 59 represents the mean of a population with brightness corrected by subtracting the binding of the second step alone. This figure illustrates that these constructs have the ability to bind CD20+ CHO cells.

EXAMPLE 40

HPLC PROFILES OF 2H7 scFv Ig MUTANT FUSION PROTEINS

HPLC analysis of purified 2H7 scFv-Ig mutant fusion proteins with altered connecting and CH3 regions. Each protein was purified by Protein A affinity chromatography from supernatants of transfected COS or CHO cells. Twenty-five to fifty micrograms of each sample was run at 1 ml/min in PBS on a TSK-GEL G3000SW_{XL} 30 cm column (Tosoh Biosep, Stuttgart, Germany). The arrow near the beginning of each profile represents the sample injection point. Gel filtration standards (Bio-Rad) included thyroglobulin (670 kDa), gamma globulin (158 kDa), ovalbumin (44 kDa), myoglobin (12.5 kDa), and vitamin B-12 (1.35 kDa). Standards were run at the beginning and end of each experiment. Migration positions of standards are shown and did not vary between experiments. (Figures 60-62)

EXAMPLE 41

BINDING CAPACITY OF 2H7 VH L11S MUTANT FUSION PROTEINS

Binding effects of the CH3 mutant were compared to the non-mutated CH3 fusion protein. The constructs were transfected into COS cells and purified from the supernatant using protein A column purification techniques described in Example 2. Figure 63 illustrates the differential effects of CH3 mutations on binding using flow cytometry according to the methods described in Example 2. This figure illustrates that some binding ability is lost when the double point mutation is introduced in the CH3 region.

Binding of fluorescein isothiocyanate (FITC) conjugated 2H7 VH L11S mutant fusion proteins was determined using flow cytometry. A 1 mg/ml solution of FITC was prepared in DMSO. Fusion proteins were dialyzed in pH 9.3 bicarbonate buffer overnight at 4C in a volume of 2 liters. Concentration of the protein was adjusted to 1-5 mg/ml prior to conjugation. A series of Falcon 5 ml tubes was set up with varying FITC to protein ratios, ranging from 15-60, but minimally ratios of 20 and 40. Conjugation reactions were incubated at 37C for 30 minutes protected from light. Fluorescing labeled

protein was separated from free fluorescein on a 2 ml Sephadex G-25 column equilibrated with PBS, 0.5 M NaCl, and 1% NaN₃. The fluorescein labeled protein eluted from the column first and was collected in a 5ml tube. The degree of labeling was determined by measuring the absorbance of the diluted conjugate at 280 and 494 nm, and utilizing the formulas provided by technical services at Molecular Probes (Eugene, OR). Data has been corrected for FITC: protein ratio. Figure 64 illustrates that these constructs do not lose binding capacity when conjugated to a fluorescent marker.

Purified 2H7 VH L11S constructs were run on a non-reducing SDS gel according to the methods described in Example 2. The migration patterns are presented in Figure 65.

EXAMPLE 42

CHARACTERIZATION OF 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3 IN Lec13 CHO CELLS

2H7 scFv VH L11S (CSC-S) H WCH2 WCH3 were transiently transfected and expressed in Lec13 CHO cells. Lec13CHO cells were used as the mammalian cell hosts for either the 2H7 scFv VHS11 hIgG1 (CSS-S)H WCH2 WCH3 and (CSC-S)H WCH2 WCH3 expressing plasmids in side-by-side transfections. All transfections were performed in 100 mm tissue culture dishes. Cells were transfected when approximately 90% confluent using lipofectamine 2000 (Invitrogen, Catalog #: 11668-027, 0.75 ml), following manufacturer's instructions. Both cell lines were grown in the presence of serum to promote and maintain adherence to the cell culture dishes, simplifying transfection manipulations, washes, and supernatant harvests. DNA: lipofectamine complexes were allowed to form in the absence of serum and antibiotics, following the suggested protocol /conditions recorded in the product insert. Culture supernatants were harvested 72 hours after transfection, and then again 72 hours after the first harvest. Fusion protein from the two CHO sources was isolated by protein A purification as previously described and used in CD20 binding and ADCC assays.

The ability of mutated fusion protein to mediate ADCC in CD20 positive cells was determined using the methods described in Example 2. Constructs expressed in Lec13 CHO cells exhibited better binding to CD20 CHO target cells and also showed significantly improved activity in ADCC assays relative to the CHO DG44 derived proteins at equivalent concentrations as illustrated in Figure 67.

EXAMPLE 43

CONSTRUCTION OF HIGH AND LOW AFFINITY CD16 ALLELES

The low(V) and high(F) affinity alleles at position 158 of the human CD16 extracellular domain were cloned from cDNA derived from human PBMC using PCR assay. PCR reactions used random primed cDNA made from PBMC stimulated for 3 days with immobilized anti-CD3 antibody (64.1) prior to harvest. PCR reactions included 2, 4, 6 or 8 microliters of cDNA, each primer at 25 pmol, and an amplification profile of 94C 60 sec; 55C 60 sec; 72C 2 min, for 35 cycles. The PCR primers are listed below:

5' primer - no leader peptide: 5'-

10 GTTGTACCGGTGCAATGCGGACTGAAGATCTCCC
AAAGGCTGTG-3' (SEQ ID NO__)

3'antisense primer: 5'-

GTTGTTTGATCAGCCAAACCTTGAGTGATGGTGATGTTTACA-3' (SEQ ID NO__)

Positive clones were sequenced, and inserted into a vector containing an efficient leader peptide and the (SSS-S)H P238SCH2 WCH3 human IgG tail. Two different versions of the CD16 ED fusion proteins were expressed. The first contained the F158 (high affinity) and the second contained the V158 (low affinity) allele. Constructs were cloned into a (SSS-S)H P238S CH2 WCH3 pD18 plasmid and expressed in COS and CHO cells as previously described in examples 1 and 10. CHO clones were screened for expression using an IgG sandwich ELISA to determine relative expression levels of the fusion proteins in the culture supernatant using the following protocol: Immulon 1V plates were coated at 4C with 0.4 microgram/ml goat anti-human IgG (mouse Adsorbed), (CalTag, Catalog # H10500) in PBS buffer. Plates were then blocked in PBS/1.5% nonfat milk at 4C overnight. Plates were washed three times in PBS/0.1% Tween 20, then incubated with 100 microliters dilution series from CHO clone culture supernatants at room temperature for 3 hours. Four dilutions per clone were added to successive wells, diluting in 5 fold increments from 1:5 to 1:375. In addition a standard curve was derived using CTLA4 hIgG1 (SSS-S)H P238SCH2 WCH3 as a concentration standard. The dilution series utilized 5 fold dilutions starting at 0.5 micrograms/ml; a second set of 8 wells was used to make a 2-fold dilution series starting at 0.34 micrograms/ml. Plates were washed 3 times in PBS/0.1% Tween 20, and incubated with goat anti-human IgG, conjugated to horseradish peroxidase (GAH IgG-HRP) at 1:5000, in PBS/0.5% BSA for 1

hour. Plates were washed four times with PBS/0.1% Tween 20, then TMB chromagen substrate (BD-Pharmingen) was added for 10 minutes, and reactions stopped by addition of 100 microlites 1N sulfuric acid. Plates were then read at 415nm on a SpectraCount plate reader. Concentrations of fusion protein were estimated by comparison of the ODs in the linear range to the CTLA4Ig standard curve run on each plate.

Proteins were purified using Protein A purification and were directly conjugated to fluorescein isothiocyanate (FITC) as described in Example 42. These proteins were run out on SDS gels under reduced and nonreduced conditions according to the methods described in Example 2. The migration of these proteins is presented in Figure 68.

The ability of the high and low CD16 alleles to bind 2H7 VH111S (CSC-S) H WCH2 WCH3 or bind 2H7 VH111S (SSS-S) H (P238S)CH2 WCH3 expressed on the cell surface of CD20+ CHO target cell is determined using flow cytometry according to the methods described in Example 2. The results in Figure 69 demonstrate both the high and low affinity alleles were able to bind 2H7 VHL11S (CSC-S)H WCH2 WCH3 (SEQ ID NO__) and lost some binding capabilities when the P238S mutation was introduced into the CH2 region of the construct (SEQ ID NO__).

EXAMPLE 44

MAMMALIAN DISPLAY SYSTEM

Figure 70A diagrams how FITC conjugates of FcRIII (CD16) soluble fusion proteins bind to 2H7 scFv-Ig constructs that are attached to CD20 expressed by CHO cells. The CD16 binding to a scFv-Ig provides a screening tool for detecting changes in CD16 binding to an altered scFv-Ig constructs containing targeted or site-specific mutations. Changes in CD16 binding properties may be changes in binding of either CD16 high affinity protein (158F) or CD16 low affinity protein (158 V) or both.

A schematic representation of such a screening process is diagrammed in Figure 70B, where scFv-Ig constructs are displayed on the cell surface of mammalian cells. The scFv-Ig molecules in this Example are displayed on the cell surface because they contain a transmembrane domain anchor. These molecules may represent a single scFv-Ig construct or may be introduced into a population of mammalian cells as a library of such molecules. Transfected cells with altered binding properties can then be panned, sorted, or otherwise isolated from other cells by altering the stringency of the selection conditions

and using CD16 fusion proteins as the binding probe. Cells that express scFv-Ig molecules with altered binding to either CD16 high affinity allele (158F) or CD16 low affinity allele (158V) or both can be isolated. For example, this display system can be used to create a library of mutated Ig tails with short stretches of CH2 sequence replaced with randomized oligonucleotides or possibly randomization of a single residue with all possible amino acid substitutions, including synthetic amino acids. Once such a library is constructed, it can be transfected into COS cells by methods well known in the art. Transfectants can then be bound to the labeled CD16 constructs, and panned or sorted based on their relative binding properties to multiple allelotypes/isoforms. Panned cells are harvested, and the plasmid DNA is isolated and then transformed into bacteria. This process may be repeated iteratively multiple times until single clones are isolated from the mammalian host cells (see Seed B and Aruffo A, PNAS 84:3365-3369 (1987) and Aruffo A and Seed B, PNAS 84: 8573-8577 (1987)). One such use of this type of screening system would be to isolate Ig tails which bind equally well to both the high and low affinity alleles of CD16 with the goal of improving effector functions mediated by scFv-Ig constructs in multiple subpopulations of patients. Ig tails with altered binding properties to other Fc receptors can also be selected using the display system described. Other display systems for example those that use bacteriophage or yeast are not suitable for selection of Ig tails with altered FcR binding properties because of the requirement for glycosylation in the Ig CH2 domain that would not occur in non-mammalian systems.

This system is also useful for selection of altered scFv-Ig molecules that will be produced at higher levels. In this Example, mammalian cells such as COS cells can be transfected with a library of scFv-Ig constructs in a plasmid that directs their expression to the cell surface. COS cells that express the highest levels of the scFv-Ig molecules can be selected by techniques well known in the art (for example panning, sterile cell sorting, magnetic bead separation, etc), and plasmid DNA is isolated for transformation into bacteria. After several rounds of selection single clones are isolated that encode scFv-Ig molecules capable of high level expression. When the isolated clones are altered to remove the membrane anchor and then expressed in mammalian cells, the scFv-Ig constructs will be secreted into the culture fluid in high levels. This reflects the common requirement of secreted glycoproteins and cell surface glycoproteins for a signal peptide and processing through the golgi for expression, so that selection for a molecule that

illustrates an improvement in expression levels on the cell surface will also select for a molecule that illustrates an improvement in levels of secreted protein.

EXAMPLE 45

CHARACTERIZATION OF G28-1 MABS AND SCFVS

5 Ability of G28-1 mAbs and scFvs to induce apoptosis was measured by binding Annexin V, using the methods described in Example 3. The results in Figure 71 demonstrate that the Annexin V binding of G28-1 antibodies and scFv is increased when treated together with 2H7 antibodies and scFv constructs.

EXAMPLE 46

CONSTRUCTION OF FC2-2 SCFV CONSTRUCTS

10 Construction of the FC2-2 (anti-CD16) scFv was performed using total RNA isolated from the FC2-2 hybridoma and cloned using methods described in Example 35. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__. The specific primers for the
15 secondary PCR reaction are listed bellow. The following are primers for the light chain variable region:

5' primer with HindIII site with no leader: 5'-

GTTGTTAAGCTTGCCGCCATGGATTCAC

AGGCCCAGGTTCTT-3' (SEQ ID NO__)

20 5' primer with SalI site and leader: 5'-

GTTGTTGTCGACATTGTGATGTCACAGTCTCC

ATCCTCCCTA-3' (SEQ ID NO__)

3' primer:

5'-TCAGTGCTGATCATGAGGAGACGGTGACTGAGGTTCCCTT-3' (SEQ ID NO__)

25 The following are primers for the heavy chain variable region:

5' primer: 5'-

TCGGGCGGTGGTGGGTCGGGTGGCGGCGGATCGTCACAGGTGCAGTTG

AAGGAGTCAGGA-3' (SEQ ID NO__)

3' primer: 5'-

30 ACCCGACCCACCAACCGCCCGAGCCACCGCCACCTTTTATTTCCAGCTTG

GTGCCACCTCCGAA-3' (SEQ ID NO__)

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the FC2-2 scFv by site-directed mutagenesis according to the methods described in Example 33. The scFv was attached to the (SSS-S) H WCH2 WCH3 IgG tail according to methods described in Example 33. The mutant is designated FC2-2 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 47

CONSTRUCTION OF 5B9 scFV CONSTRUCTS

Contrstruction of the 5B9 (anti-CD137) scFv was performed using total RNA isolated from the 5B9 hybridoma and cloned using methods described in Example 35. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__. The specific primers for the secondary PCR reaction are listed bellow. The following are primers for the light chain variable region:

5' primer with HindIII site with no leader: 5'-

GTTGTTAAGCTTGCCGCCATGAGGTTCT

CTGCTCAGCTTCTG-3' (SEQ ID NO__)

5' primer with SalI site and leader: 5'-

GTTGTTGTCGACATTTGTGATGACGCAGGCTG

CATTCTCCAATT-3' (SEQ ID NO__)

3' primer: 5'-TCAGTGCTGATCAGAGGAGGACGGTGACTGAGGTTTCCTTG-3' (SEQ ID NO__)

The following are primers for the heavy chain variable region:

5' primer: 5'-

CGGGCGGTGGTGGGTCGGGTGGCGGCGGATCGTCACAGGTGCAGCTGA

AGCAGTCAGGA-3' (SEQ ID NO__)

3' primer: 5'-

CCCGACCCACCACCGCCCGAGCCACCGCCACCCCTTCAGCTCCAGCTTG

GTGCCAGCACC-3' (SEQ ID NO__)

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the 5B9 scFv by site-directed

mutagenesis and attached to (SSS-S) H WCH2 WCH3 according to the methods described in Example 33. This construct was designated 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

5

EXAMPLE 48**CONSTRUCTION OF UCHL1 scFv CONSTRUCTS**

Contrstruction of the UCHL1 (anti-CD45RO) scFv was performed using total RNA isolated from the UCHL1 hybridoma and cloned using methods described in Example 35 The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__. The following are primers for the light chain variable region:

5' primer with HindIII site: 5'-

GTTGTTAAGCTTGCCGCCATGAAGTTGCCTGTTAGGCTG

TTGGTGCTG-3' (SEQ ID NO__)

15 3' primer with Sac site: 5'-

AGAGCTCCACCTCCTCCAGATCCACCACCGCCCGAGCCAC

CGCCATCTTTGATTTCCAGCTTGGT-3' (SEQ ID NO__)

The following are primers for the heavy chain variable region:

5' primer: 5'-

20 TTTCAGAGTAATCTGAGAGCTCCACCTCCTCCAGATCCACCACCGC

CCGA-3' (SEQ ID NO__)

3' primer: 5'-TCAGTGCTGATCATGCAGAGAGACAGTGACCAGAGTCCC-3' (SEQ ID NO__)

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the 5B9 scFv by site-directed mutagenesis and connected to a(SSS-S) HWCH2 WCH3 according to the methods described in Example 33. The mutant is designated UCHL1 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 49**L6 VHL11S scFv (SSS-S) H WCH2 WCH3**

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the L6 scFv (SSS-S) H WCH2 WCH3 (constructed according to methods described in Example 106) by site-directed mutagenesis according to the methods described in Example 33. The L6scFvIg (SSS-S) H WCH2 WCH3 pD18 plasmid was used as template. Positive clones were inserted into the pD18 plasmid containing (SSS-S) H WCH2 WCH3 according to methods described in Example 33. The mutant is designated L6 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

10 PCR primers are listed bellow:

5' Primer with PstI restriction site:5'-

ggcggatctctgcagatccagttggtgcagtctggacctgagtcgaagaagcct

ggagag-3' (SEQ ID NO __)

3'Primer: 5'-ggacagtgggagtggcacc-3' (SEQ ID NO __)

15

EXAMPLE 50

CONSTRUCTION OF HD37 scFv VHL11S CONSTRUCT

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the HD37 scFv by site-directed mutagenesis according to the methods described in Example 33. The HD37 scFv (SSS-S)H WCH2 WCH3 pD18 plasmid was used as a template. Positive clones were inserted into the pD18 plasmid containing (SSS-S) H WCH2 WCH3 according to methods described in Example 33. The mutant is designated HD37 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__. PCR primers are listed bellow:

25

5' primer: 5'-caggttcagctgcagcagctctggggctgagtcggtgaggcctgg-3' (SEQ ID NO __)

3' primer: 5'-ggaggattcgtctgcagtcagagtggc-3' (SEQ ID NO __)

EXAMPLE 51

2H7 scFv VHL11S CONSTRUCTS

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Additional 2H7 VHL11S constructs were made with different connecting regions. The pD18 2H7 scFv VHL11S (SSS-S) H WCH2 WCH3 vector was digested with

BclI and XbaI to remove the connecting region, CH2 and CH3. These were replaced with each different connecting region, CH2 and CH3 according to the methods described in Example 13. The new constructs were designated: 2H7scFv VHL11S (CSS-S) H WH2 WH3 (SEQ ID NO___), 2H7scFv VHL11S (CSC-S) H WH2 WH3 (SEQ ID NO___). 2H7 scFv VHL11S was also attached to an IgA connecting region, CH2, CH3 and an IgE CH2, CH3, CH4. The pD18 2H7 scFv VHL11S (SSS-S) H WCH2 WCH3 plasmid was digested using methods above to remove the connecting region CH2, and CH3. The IgA regions were inserted using methods described in Example 13. The construct was designated 2H7 scFv VHL11S IgAH IgACH2 T4CH3 (SEQ ID NO___). The IgE CH2 CH3 CH4 region was inserted into the digested pD18 vector above using methods described in Example 39. The construct was designated 2H7 scFv VHL11S IgECH2 CH3 CH4 (SEQ ID NO___).

EXAMPLE 52

2H7 scFv VH L11S (CSC-S) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a human IgG1 connecting region, CH2 and CH3 region, where in the second cysteine and the proline in the connecting region have been changed to serines (SSS-S) as described in Example 32. The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 53

2H7 scFv VH L11S IgE CH2 CH3 CH4

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 54

2H7 scFv VH L11S mIgE CH2 CH3 CH4

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a mouse IgE constant region containing CH2, CH3 and CH4 as described in
 5 Example 39. . The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 55

2H7 scFv VH L11S mIGHA1 WIGACH2 T4CH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a
 10 point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a mouse IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues
 15 prior to the 3' stop codon as described in Example 39. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 56

2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example
 25 5. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322S mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a serine using overlapping PCR assay. An (SSS-S) H WCH2 WCH3 IgG1 template in the pD18 vector was used for PCR amplification, to create (SSS-S) H derivatives containing these CH2 mutations. PCR reactions used a cycling profile of 94C,
 30 30 sec; 55C, 30 sec; 72C, 30 sec, for 37 cycles to complete the reactions. This IgG1 derivative was constructed by using sequential PCR reactions with overlapping

oligonucleotides in the primary and secondary reactions. The primary amplification primers introduced the mutation(s), but deleted one end of the Fc domain. Secondary reaction primers reattached these ends using overlapping primers. The first overlapping primer was added at the beginning of the PCR, the reactions allowed to proceed for 12 cycles, paused and then the second overlapping primer added to the reactions followed by 25 more cycles to complete the overlap extension PCR reactions.

Primers for the first PCR reaction:

5' Primer: 5'-ggagatggtttctcgatgggggctgggagggttggtggagaccgagcactgtactcc-3' (SEQ ID NO)

10 3' primer: 5'-ggacagtgggagtggcacc-3' (SEQ ID NO)

PCR products were cloned into TOPO vector and sequenced for verification. Positive vectors were used as templates for the second overlapping PCR reaction.

15 5' primer: 5'ccgtctctgatcaggagcccaaattctctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

5' primer overlapping primer: 5'-

tccccaccgtccccagcacctgaactcctgggggatcgctcagtcttcttcttcccccaaaacc-3' (SEQ ID NO)

3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO)

20 PCR product was cloned into TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 57

2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3

25 This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a mutant IgG connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322S mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a serine using overlapping PCR assay.

region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The mutation in the CH2 region was added by overlapping PCR reaction essentially according to Example 57, with (CSS-S) H WCH2 WHC3 IgG1 pD18 vector as a template in the first PCR reaction and different primers in the second PCR reaction, which are listed below.

5 5'primer: 5'- ccgtctctgatcaggaccccaaatcttgtgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO__)

5'overlapping primer: 5'-

tccccaccgtccccagcacctgaactcctggggggatcgtagcttcttcttcccccaaaacc-3'

3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO)

10 PCR products were cloned into the TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 58

2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3

15 This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 20 5. The connecting region is attached to a mutated IgG1 CH2 region and a wild type IgG1 CH3 region. The mutation P331S mutation in the CH2 region, where the proline at residue 331 has been changed to a serine, was incorporated using a single PCR reaction, using a (SSS-S) H WCH2 WCH3 pD18 template and a cycling profile of 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, for 37 cycles. The specific primers for reaction are listed below.

25 5' primer: 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

3' Primers: 5'-
gcagggtgtacacctgtggttctcggggctgccctttggctttggagatggtttctcgatggaggctgggagg-3' (SEQ ID NO)

30 The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 59**2H7 scFv VH L11S (CSS-S) H P331S CH2 WCH3**

This binding region is attached to a mutant IgG connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The mutation P331S mutation in the CH2 region, where the proline at residue 331 has been changed to a serine, was incorporated using a single PCR reaction, using a (CSS-S) H WCH2 WCH3 pD18 template and a cycling profile of 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, for 37 cycles. The specific primers for reaction are listed below.

5' primer: 5'-ccgtctctgatcaggaccccaaatctgtgacaaaactcacatccccaccgtccccagc-3' (SEQ ID NO)

3' Primer: 5'-

gcagggtgtacacctgtggttctcggggctgcccttggcttggagatggtttctcgatggaggctgggagg-3' (SEQ ID

NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 60**2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The T256N mutation in the CH2 region, the threonine at residue 256 has been changed to an asparagine, using the overlapping PCR methods described in Example 56. The specific primers are listed below.

Primers for the first PCR reaction:

5' primer: 5'ttctcttcccccaaaaccaaggacaccctcatgatctcccgaaccctgaggtcac-3' (SEQ ID NO)

3' primer: 5'-ggacagtgggagtggcacc-3' (SEQ ID NO)

PCR product cloned into TOPO vector and sequenced. This product was used as the template in the second PCR reaction. Primers for the second PCR reaction:
 5' primer: 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

- 5 5'overlapping primer: 5'-
 tccccaccgtccccagcacctgaactcctggggggatcgctcagtcttctcttccccccaaaacc-3' (SEQ ID NO)
 3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 61

2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3

- This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is
 15 connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The RTPE/QNAK mutation in the CH2 region, where residues 255-258 have
 20 been mutated from arginine, threonine, proline, glutamic acid to glutamine, asparagines, alanine and lysine, respectively, using the overlapping PCR reactions described in Example 56. The specific primers are listed below.

PCR primers for the first PCR reaction:

- 5' primer: 5'-ttctcttccccccaaaaccaaggacaccctcatgatctccagaacgctaaggtcacatgc-3' (SEQ ID NO __)
 25 3' primer: 5'-ggacagtgggagtggcacc-3' (SEQ ID NO)

The PCR product was cloned into TOPO vector, sequenced and used as a template for the second PCR reaction. The primers for the second PCR reaction:

- 5 primer: 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)
 30 5'overlapping primer:5'-
 tccccaccgtccccagcacctgaactcctggggggatcgctcagtcttctcttccccccaaaacc-3' (SEQ ID NO)
 3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 62

2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3

5 This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example

10 5. The connecting region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The K290Q mutation in the CH2 region, where the Lysine at residue 290 has been changed to a Glutamine, using a single PCR reaction according to the methods described in Example 58. The specific primers used in this reaction are listed below.

15 5' primer: 5'-ccgtctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtccccagc-3' (SEQ ID NO)

3' primer: 5'-gctcccgcggtgtgtcttggc-3' (SEQ ID NO)

PCR products were cloned into TOPO and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided

20 in SEQ ID NO:___.

EXAMPLE 63

2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the

25 leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The A339P mutation in the CH2 region, where the alanine

30 at residue 339 has been changed to a proline, using a single PCR reaction according to the

methods described in Example 58. The specific primers used in this reaction are listed below.

5' primer: 5'-ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

5 3' Primer: 5'-ggaggtgggcagggtgtacacctgtggttctcggggctgcccttgggttggagatgg-3' (SEQ ID NO)

PCR products were cloned into TOPO and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 64

G28-1 scFv (SSS-S) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region made according to methods described in Example 35. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have
15 been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. This construct has previously been referred to as G28-1-MHWTG1C and G28-1 scFv Ig, both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide
20 sequence is provided in SEQ ID NO:__.

EXAMPLE 65

G28-1 scFv IgAH WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region made according to methods described in Example 35. This binding region is connected to
25 a human IgA connecting region and wild type human IgG CH2 and CH3 constant regions as described in Example 5. This construct has previously been referred to as: G28-1-IgAHWTG1C. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 66

G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3

30

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:_____.

EXAMPLE 67

G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S); according to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:_____.

EXAMPLE 68

G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the second cysteine and the proline has been changed to serine (CSC-S), according to methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:_____.

EXAMPLE 69

G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the first and second cysteines have been changed to serines (SSC-P), according to methods described in Example 13. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 70

CTLA4 (SSS-S) H P238SCH2 WCH3

This construct has the extra cellular CTLA-4 binding region as described in Example 14. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced using a PCR assay. PCR reactions were performed using random primed cDNA prepared from human tonsil B cell RNA. PCR amplifications used an amplification profile of 94C 4 min; [94C 1 min; 55C 1 min; 72C 1min; for 30 cycles followed by a final extension step for 6 minutes at 72C. PCR fragments were TOPO cloned and clones with EcoRI inserts of approximately 800 bp were sequenced as described in Example 1. The primers used for the PCR are listed below:

5' primer: 5'-

gttgttgatcaggagcccaaatcttctgacaaaactcacacatctccaccgtccccagcacctgaactcctgggtgg
accgtcagtcttcc-3' (SEQ ID NO)

3' primer: 5'gttggttctagattatcatttaccggagacag-3' (SEQ ID NO)

This construct has previously been referred to as CTLA-4 IgG MTH (SSS) MTCH2WTCH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 71

CTLA4 (CCC-P) WH WCH2 WCH3

This construct has a CTLA-4 binding region as described in Example 14. This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. This construct has previously been referred to as CTLA-4 IgG WTH (CCC) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 72

FC2-2 scFv (SSS-S) H WCH2 WCH3

This construct has a FC2-2 (anti-CD16) single chain Fv made according to methods described in Example 46. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 73

FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3

This construct has a FC2-2 (anti-CD16) single chain Fv with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 46. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 74

UCHL-1 scFv (SSS-S) H WCH2 WCH3

This construct has a UCHL-1 (anti-CD45RO) single chain Fv made according to methods described in Example 48. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have

been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 75**UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3**

This construct has a UCHL-1 (anti-CD45RO) single chain Fv with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 48. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 constant region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

15

EXAMPLE 76**5B9 scFv (SSS-S) H WCH2 WCH3**

This construct has a 5B9 (anti-CD137) single chain Fv made according to methods described in Example 47. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 constant region as described in Example 1. This construct has previously been referred to as 5B9 scFv IgG MTH (SSS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

25

EXAMPLE 77**5B9 scFv VHL11S (SSS-S) H WCH2 WCH3**

This construct has a 5B9 (anti-CD137) single chain Fv with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 47. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have

30

been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 constant region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 78**2H7 scFv (SSS-S) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type IgG1 CH2 and CH3 constant region as described in Example 1. This construct has previously been referred to as 2H7-MHWTG1C, CytoxB-(MHWTG1C) – Ig, anti-CD20 scFv IgG MTH (SSS) WTCH2CH3, CytoxB-MHWTG1C, 2H7 scFv-human IgG1 wild type hinge-CH2-CH3, and 2H7 scFv IgG MTH (SSS) WTCH2CH3, which all have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 79**2H7 scFv (SSS-S) H P238SCH2 WCH3**

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This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This construct has previously been referred to as 2H7 scFv IgG MTH (SSS) MTCH2WTCH3, anti-CD20 scFv IgG MTH (SSS) MTCH2CH3, and CytoxB-MHMG1C which all have the same

sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 80

2H7 scFv IgAH WCH2 WCH3

5 This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a human IgA connecting region and wild type human IgG1 CH2 and CH3 constant regions as described in Example 5. This construct has previously been referred to as 2H7 scFv IgAH IgG WTCH2CH3, 2H7 scFv IgA hinge-IgG1 CH2-CH3, and CytosB-IgAHWTHG1C, which all have the
10 same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 81

2H7 scFv IgAH WIGACH2 T4CH3

15 This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. This construct has previously been referred to as 2H7 scFv IgAH IgAT4,
20 which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 82

2H7 scFv IgAH WIGACH2 WCH3 + JCHAIN

25 This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a wild type human IgA connecting region as described in Example 5. This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13. This constant region is attached to a J-chain region as described in Example 13. The
30 polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 83**2H7 scFv (CCC-P) WH WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv Ig WTH (CCC) WTCH2CH3, 2H7 scFv IgG WTH WTCH2CH3, and 2H7 scFv-Ig, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 84**2H7 scFv (SSS-S) H WCH2 F405YCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The F405Y mutation, where the phenylalanine at residue 405 has been changed to a tyrosine, was introduced according to methods described in Example 21. This construct has previously been referred to as 2H7 scFv MTH WTCH2 MTCH3 Y405, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 85**2H7 scFv (SSS-S) H WCH2 F405ACH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The F405A mutation, where the phenylalanine at residue 405 has been changed to an alanine, was introduced according to methods described in Example 21. This construct has previously

been referred to as 2H7 scFv MTH WTCH2 MTCH3 A405, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 86

5 2H7 scFv (SSS-S) H WCH2 Y407ACH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached
10 to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The Y407A mutation, where the tyrosine at residue 407 has been changed to an alanine, was introduced according to methods described in Example 21. This construct has previously been referred to as scFv MTH WTCH2 MTCH3 A407, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the
15 encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 87

2H7 scFv (SSS-S) HWCH2 F405A, Y407ACH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as
20 described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S), according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The F405A and Y407A mutation, where the phenylalanine at residue 405 has been changed to an alanine
25 and the tyrosine at residue 407 has been changed to an alanine, was introduced according to methods described in Example 21. This construct has previously been referred to as scFv MTH WTCH2 MTCH3 A405A407, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 88

2H7 scFv (CSS-S) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (CSS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

Example 89

2H7 scFv (SCS-S) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the first and third cysteines have been changed to serines and the proline has been changed to serine (SCS-S), according to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv IgG MTH (SCS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 90

2H7 scFv (SSC-P) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the first and second cysteines have been changed to serines (SSC-P), according to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (SSC) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 91**2H7 scFv (CSC-S) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the second cysteine and the proline has been changed to serine (CSC-S), according to methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (CSC) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 92**2H7 scFv (CCS-P) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where third cysteine has been changed to a serine (CCS-P), according to methods described in Example 22. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (CCS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 93**2H7 scFv (SCC-P) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where first cysteine has been changed to a serine (SCC-P), according to methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (SCC) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: __, and the encoded polypeptide sequence is provided in SEQ ID NO: __.

EXAMPLE 94**2H7 scFv VH L11S (SSS-S) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv VH11SER IgG MTH (SSS) WTCH2CH3 and 2H7 scFv VH11SER11 WTH WTCH2CH3, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 95**2H7 scFv VH L11S (CSS-S) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 33. This binding region is attached to a mutant human IgG1 connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

Example 96**G28-1 scFv VH L11S (SCS-S) H WCH2 WCH3**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the first and third cysteines have been changed to serines and the proline has been changed to serine (SCS-S),

according to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 97**G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where third cysteine has been changed to a serine (CCS-P), according to methods described in Example 22. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 98**G28-1 scFv VH L11S (SCC-P) H WCH2 WCH3**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where first cysteine has been changed to a serine (SCC-P), according to methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 99**G28-1 scFv VH L11S mIgE CH2 CH3 CH4**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a wild type mouse IgE CH2 CH3 and CH4 region using methods described in

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Example 39. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 100

G28-1 scFv VH L11S MIGA H WIGACH2 T4CH3

5 This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a connecting region from mouse IgA as described in Example 39. This connecting region is attached to a mouse IgA constant region consisting of a wild type
10 CH2 region and a mutated CH3 region where there is a 4 amino acid truncation at residues as described in Example 39. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 101

G28-1 scFv VH L11S HIGE CH2 CH3 CH4

15 This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a wild type human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. The polynucleotide sequence is provided in SEQ ID NO:__,
20 and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 102

G28-1 scFv VH L11S HIGA H WIGACH2 T4CH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the
25 leucine has been changed to a serine as described in Example 35. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. The polynucleotide sequence is
30 provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 103

HD37 scFv IGAH WCH2 WCH3

The HD37 scFv was cloned from the HD37 hybridoma using the Novagen – Ig family primer sets, TOPO cloning and sequencing and sewing PCR assay. For the
 5 initial PCR reactions prior to sewing, the TOPO clone templates HD37 VH C-1 and HD37 KVL B-9 were used at 1:100 with an amplification profile of 94C 30 sec; 55C, 30 sec; 72C, 30 seconds for 25 cycles. To provide templates for the secondary SEWING PCR reactions, primary reaction products were gel purified, QIAQUICK purified, and the eluates diluted 50 fold. One microliter each VL and VH overlapping templates were added to PCR
 10 reactions with the following amplification profile: 94C, 60 sec; 55C, 60 sec; 72C, 60 sec; for 30 cycles. After two cycles, the machine was paused, and the flanking 5'VL and 3'VH primers were added to the reactions at 25 pmol each, and the PCR reactions resumed. PCR products were checked on a gel for the presence of an 750-800 bp fragment, and the reactions products QIAQUICK purified and digested with the appropriate restriction
 15 enzymes for insertion into pD18 Ig expression vectors.

PCR of VL domain with native leader peptide and part of glyser linker:

5' primer: 5'-gttgtaagcttgccgccatggagacagacacactcctgctatgg-3' (SEQ ID NO)

3' primer: 5'-gccacccgacccaccaccgcccagccaccgccacctttgattccagcttggtgcctcc-3' (SEQ ID NO)

20 PCR of VL domain without leader peptide (SalI site) and part of glyser linker:

5' primer: 5'-gttggtgtcgacattgtgctgacccaatctcca-3' (SEQ ID NO)

3' priemr: 5'-gccacccgacccaccaccgcccagccaccgccacctttgattccagcttggtgcctcc-3' (SEQ ID NO)

25 PCR of VH domain with part of glyser linker and BclI site for fusion to –Ig tails.

5'primer: 5'-tcgggcggtggtgggtcgggtggcggcgatcgtcacagggtcagctgcagcagctctgg-3' (SEQ ID NO)

3' primer: 5'-tcagtgtgatcagaggagacgggtgactgaggttccttg-3' (SEQ ID NO)

30 This binding region is connected to a wild type human IgA connecting region and wild type human IgG CH2 and CH3 constant regions as described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant

regions as described in Example 1. This construct has previously been referred to as HD37 scFv-IgAHWTG1C and HD37- IgAHWTG1C, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 104**HD37 scFv (SSS-S) H WCH2 WCH3**

This construct has a HD 37 single chain Fv as described in Example 103. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as HD37- MHWTG1C and HD37 scFv-IgMHWTG1C, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 105**HD37 scFv VH L11S (SSS-S) H WCH2 WCH3**

This construct has a HD 37 single chain Fv with a mutation in the heavy chain variable region at amino acid residue 11, where leucine has been changed to serine according to the methods described in Example 50. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

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EXAMPLE 106**L6 scFv IGAH WCH2 WCH3**

The L6 scFv was cloned from the L6 hybridoma (I Hellstrom) using the anchor-tailing method described in the Tissue Antigens Paper from 1996. The PCR profile was 94C, 1 min; 50C, 2 min; 72C, 2 min; for 35 cycles. Once consensus sequence was obtained for VL and VH regions from at least 4 TOPO clones, primers were ordered for PCR reactions prior to SEWING PCR reactions as follows: For the initial PCR reactions

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prior to sewing, the TOPO cloned templates L6VK and L6VH were used at 1:100 with an amplification profile of 94C 30 sec; 55C, 30 sec; 72C, 30 seconds for 25 cycles. To provide templates for the secondary SEWING PCR reactions, primary reaction products were gel purified, QIAQUICK purified, and the eluates diluted 50 fold. One microliter each VL and VH overlapping templates were added to PCR reactions with the following amplification profile: 94C, 60 sec; 55C, 60 sec; 72C, 60 sec; for 30 cycles. After two cycles, the machine was paused, and the flanking 5'VL and 3'VH primers were added to the reactions at 25 pmol each, and the PCR reactions resumed. PCR products were checked on a gel for the presence of an 750-800 bp fragment, and the reactions products QIAQUICK purified and digested with the appropriate restriction enzymes for insertion into pD18 Ig expression vectors.

PCR of VL domain with native leader peptide and part of glyser linker:

L6VLHindIII: 5'-gttgtaagcttgccgccatggatttcaagtcagatttcagcttc-3' (SEQ ID NO)

L6VLLK3: 5'-gccacccgacccaccaccgcccagccaccgcccagagagctcttcagctccagcttggt-3'

(SEQ ID NO)

PCR of VL domain without leader peptide (SalI site) and part of glyser

linker:

5' primer: 5'-gttggtgtcgacattgttctctccagctctccagcaatcctgtctg-3' (SEQ ID NO)

3' primer: 5'-gccacccgacccaccaccgcccagccaccgcccagagagctcttcagctccagcttggt-3'

(SEQ ID NO)

PCR of VH domain with part of glyser linker and BclI site for fusion to -Ig tails.

5': 5'-tcgggcggtgggtgggtcgggtggcgcgatctctgcagatccagttggtgcagtct-3' (SEQ ID NO)

3'Bcl: 5'-tcagtgtgatcagaggagactgtgagagtgggtgccttg-3' (SEQ ID NO)

This binding region is connected to a human IgA connecting region and wild type human IgG1 CH2 and CH3 constant regions as described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as L6 scFv-IgAHWTG1C, which HAS the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 107

L6 scFv VHL11S (SSS-S) H WCH2 WCH3

This construct has a L6 single chain Fv with a mutation in the heavy chain variable region at amino acid residue 11, where leucine has been changed to serine according to the methods described in Example 49. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 108**2H7 scFv-LLAMA IGG1**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a llama IgG1 hinge, CH2 and CH3 regions according to the methods described in Example 10. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 109**2H7 scFv-LLAMA IGG2**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a llama IgG2 hinge, CH2 and CH3 regions according to the methods described in Example 10. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 110**2H7 scFv-LLAMA IGG3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a llama IgG3 hinge, CH2 and CH3 regions according to the methods described in Example 10. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 111**CD16 LOW (ED)(SSS-S) H P238SCH2 WCH3**

This construct has the extra cellular, CD16 low affinity allele binding domain as described in Example 43. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 112**CD16-9 HIGH (ED)(SSS-S) H P238SCH2 WCH3**

This construct has the extra cellular, CD16 high affinity allele binding domain as described in Example 43. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

Example 113**2e12 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT**

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a human

CD80 transmembrane and cytoplasmic tail region (hCD80 TM/CT). The hCD80 TM/CT was cloned using random primed cDNA derived from the BJAB cell line according to methods described in Example 12. This TM/CT region was attached to a Ig CH3 region with an open reading frame (ORF). Open reading frame versions of scFvIg constructs of interest were created by replacement of the soluble versions of each -Ig tail with ORF (open reading frame versions) of these tails. PCR primers were designed for the existing clones of soluble -Ig tails which delete the stop codon and add one or more restriction sites to the 3' end of the new -Ig cassettes. The desired transmembrane and cytoplasmic tail sequences can then be subcloned downstream of these new -Ig cassettes. Each construct utilized the existing available 5' BCLI oligonucleotide used in amplifying the soluble version of the tails for the PCR reactions. The 3' oligonucleotides replace the stop codon with out of frame restriction sites fused to the coding region for protein domains involved in regulation of apoptosis.

The PCR amplifications were carried out with 25 pmol of each primer, standard PCR reagents, and varying volumes of either cloned domains or cDNA obtained from PBMC, spleen, or thymus RNA. The reactions used a cycling profile of 94C, 60sec; 55C, 60 sec; 72C, 2 min, for 35 cycles. The primers for the IgG ORF are listed below.

5' primer: 5'-

gtttagatcaggagcccaaatcttctgacaaaactcacacatctccaccgtccccagcacctgaactcctggg

ggaccgtcagtccttcc-3'(SEQ ID NO)

3' primer: 5'-gttgttttcgaaggatccgctttaccgggagcagggagaggctcttctgcgtgtagtg-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 114

10A8 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a 10A8 (anti-CD2152) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according

to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 115

40.2.220 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a 40.2.220 (anti-CD40) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 116

2H7 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S), according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. This construct has previously been referred to as: The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 117

G19-4 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a G19 (anti-CD3) single chain Fv binding region described in Example 29. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines

(SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 118

2E12 scFv (SSS-S)H WCH2 WCH3—hCD80TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. This construct has previously been referred to as 2e12 scFv IgG WTH WHTCH3CH2-CD80, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 119

2E12 scFv IgAH IgACH2 T4CH3—hCD80TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. The specific primers used to create an IgA ORF are listed below.

5' primer: 5'-gttggtgatcagccagttccctcaactccacctacc-3' (SEQ ID NO)

3' primer: 5'-gttggtttcgaaggatccgcgtccacctccgccatgacaacaga (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 120

2E12 scFv IgE CH2CH3CH4—hCD80TM/CT

5 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. This CH4 region is attached to a hCD80 TM/CT essentially according to methods described in Example 113. The specific primers used to create an IgE ORF are listed below.

10 5' primer: 5'-gttgtgatcacgtctgctccaggacttcacc-3' (SEQ ID NO)

3'primer: 5'-gttgtttcgaaggatccgctttaccagattacagacaccgctcgtg-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:___, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 121

2E12 scFv (SSS-S)H P238SCH2 WCH3—mFADD-TM/CT

15 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a
20 mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a mouse FADD transmembrane and cytoplasmic tail region (mFADD TM/CT). This region is cloned using
25 from randomly primed cDNA from mouse spleen RNA. The specific primers are listed below.

5' primer: 5'-gttgtgatccttcgaaccattcctggtgctgctgcactcgtg-3' (SEQ ID NO)

3' primer: 5'-gttgttatcgatctcgagtcagggtgttctgaggaagacacagt-3' (SEQ ID NO)

The specific primers used to create a mouse IgG ORF are listed below.

30 5'primer: 5'-gtttagatctggagcccagaggcccacaaatcaagccctctcctcaagcaaaagccca-3' (SEQ ID NO)

3' primer: 5'-gtgttttcgaaggatccgctttacccggagtcgaggagaag-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 122

5 2E12 scFv (SSS-S)H WCH2 WCH3—mFADD-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached
10 to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 123

15 2E12 scFv (SSS-S)H WCH2 WCH3—mCASP3-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached
20 to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a mouse casp3 transmembrane and cytoplasmic tail region according to methods described in Examples 113 and 121. The specific primers used to isolate the mcasp3 TM/CT region are listed below:

5' primer: 5'-gtgttgatccttcgaacatggagaacaacaaacctcagtggattca-3' (SEQ ID NO)

25 3' primer: 5'-gtgttatcgatctcgagctagtataaaagtacagttcttcgt-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:___.

EXAMPLE 124

30 2E12 scFv (SSS-S)H P238SCH2 WCH3—mCASP3-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1

connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is connected to a mcasp3 TM/CT region according to methods described in Examples 113, 121 and 123. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 125

2E12 scFv (SSS-S)H WCH2 WCH3—MCASP8—TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a mouse casp8 transmembrane and cytoplasmic tail region (mcasp8 TM/CT) essentially according to methods described in Example 113 and 121. The specific primers used to clone the mcasp8 TM/CT region are listed below.

5' primer: 5'-gtgtttcgaacatggattccagagttgtctttatgctattgctg-3' (SEQ ID NO)

3' primer: 5'-gtgttatcgatctcgagtcattagggagggaagaagagcttctccg-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 126

2E12 scFv (SSS-S)H P238SCH2 WCH3—MCASP8-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mcasp8 TM/CT

region according to methods described in Examples 113, 121 and 125. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 127

2e12 scFv (SSS-S)H WCH2 WCH3—HCASP3—TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region was attached to a human casp3 transmembrane and cytoplasmic tail region (hcasp3 TM/CT) essentially according to methods describe in Examples 113. The specific primers used to clone the hcasp3 TM/CT region are listed below.

5' Primer: 5'-gttgtggatccttcgaacatggagaacactgaaaactcagtggat-3' (SEQ ID NO)

3' Primer: 5'-gttggtatcgatctcgagtttagtataaaaatagagttctttgtgag-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 128

2e12 scFv (SSS-S)H P238SCH2 WCH3—HCASP3-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hcasp3 TM/CT region according to methods described in Examples 113 and 127. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 129

2e12 scFv (SSS-S)H WCH2 WCH3—HCASP8--TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a human casp8 transmembrane and cytoplasmic tail region (hcasp8 TM/CT) according to methods described in Example 133. The specific primers used to clone the hcasp8 TM/CT region are listed below.

5' Primer: 5'-gttggtgatccttcgaacatggacttcagcagaaatctttatgat-3' (SEQ ID NO)

10 3' Primer: 5'-gttggtatcgatgcagctcaatcagaagggaagacaagtttttct-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 130

2e12 scFv (SSS-S)H P238SCH2 WCH3—HCASP8-TM/CT

15 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S
20 mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

25

EXAMPLE 131

1D8 scFv (SSS-S)H P238SCH2 WCH3—HCD80TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines
30 (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S

mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hCD80 TM/CT region according to methods described in Example 113. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 132

1D8 scFv (SSS-S)H WCH2 WCH3—hCD80TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a hCD80 TM/CT region according to methods described in Example 113. This construct has previously been referred to as 1D8 scFv IgG WTH WTCH2CH3-CD80, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 133

1D8 scFv—MIGA H WIGA CH2 T4CH3—hCD80TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a mouse IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 39. The CH3 region can be attached to a hCD80 TM/CT region according to methods described in Examples 113 using primers that create an IgA ORF.

EXAMPLE 134

1D8 scFv IgE CH2CH3CH4 -hCD80TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. The CH4 region is attached to

a hCD80 TM/CT according to methods described in Examples 113 and 120. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:_____.

EXAMPLE 135

5 1D8 scFv (SSS-S)H P238SCH2 WCH3—mFADD-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a
10 mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided
15 in SEQ ID NO:_____.

EXAMPLE 136

1D8 scFv (SSS-S)H WCH2 WCH3—mFADD-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1
20 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121. The polynucleotide sequence is provided in SEQ ID NO:__, and
25 the encoded polypeptide sequence is provided in SEQ ID NO:_____.

EXAMPLE 137

1D8 scFv (SSS-S)H WCH2 WCH3—mcasp3-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1
30 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached

to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region was attached to a mcasp3 TM/TM region according to methods described in Example 113, 121 and 123. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

5

EXAMPLE 138**1D8 scFv (SSS-S)H P238SCH2 WCH3—MCASP3-TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mcasp3 TM/TM region according to methods described in Example 113, 121 and 123. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 139**1D8 scFv (SSS-S)H WCH2 WCH3—MCASP8--TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region was attached to a mcasp8 TM/TM region according to methods described in Example 113, 121 and 125. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 140**1D8 scFv (SSS-S)H P238SCH2 WCH3—MCASP8-TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines

(SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mcasp8 TM/TM region according to methods described in Example 113, 121 and 125. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 141

1D8 scFv (SSS-S)H WCH2 WCH3—HCASP3--TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a hcasp3 TM/CT according to methods described in Examples 113 and 127. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 142

1D8 scFv (SSS-S)H P238SCH2 WCH3—HCASP3-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hcasp3 TM/CT according to methods described in Examples 113 and 127. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 143

1D8 scFv (SSS-S)H WCH2 WCH3—HCASP8--TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 144

1D8 scFv (SSS-s)H P238SCH2 WCH3—HCASP8-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 145

L6 scFv (SSS-S) H WCH2 WCH3

This construct has a L6 scFv binding domain as described in Example 105. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as L6 scFv-IgMHWGTG1C, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 146

2H7 scFv CD154 (L2)

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region has been attached to CD154 extracellular domain according to methods described in Example 4. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 147**2H7 scFv CD154 (S4)**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region has been attached to CD154 extracellular domain according to methods described in Example 4, such that methods of attachment resulted in a truncated version compared to the construct describe in Example 146. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 148**CTLA4 IgAH IgACH2CH3**

This construct has the extra cellular CTLA-4 binding region as described in Example 14. This binding region is attached to a wild type human IgA connecting region as described in Example 5. This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13. This constant region is attached to a J-chain region as described in Example 13. This construct has previously been refered to as CTLA-4 IgAH IgACH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 149**CTLA4 IgAH IgACH2 T4CH3**

This construct has the extra cellular CTLA-4 binding region as described in Example 14. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. This

construct has previously been referred to as CTLA-4 IgAH IgA-T4, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 150

2H7 scFv IgAH IgACH2CH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a wild type human IgA connecting region as described in Example 5. This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 151

2H7 scFv IgAH IgAHCH2 T18CH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 18 amino acid residues prior to the 3' stop codon as described in Example 13. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 152

2H7-40.2.220 scFv (SSS-S) H WCH2 WCH3

A bispecific fusion protein was constructed between 2H7 scFv (anti-CD20) and 40.2.220 (anti-CD40), which both target B cell receptors. The 2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3 construct in the expression vector pD18 was passaged through dam^r bacteria in order to permit cleavage at the BclI site. Cleaved plasmid was treated with alkaline phosphatase prior to ligation to a BclI cut linker-CD40 scFv fragment. This fragment was synthesized from the existing 40.2.220 scFv by successive PCR reactions with overlapping primers. The linker attached is a previously patented (BMS patent issued) helical type linker with a high number of lysine and glutamic acid residues. The scFv for CD40 was PCR amplified without the leader peptide as a SalI-BclI fragment, but

tail included in an existing scFvIg construct for CD20 (2H7 scFv hlgG1 constructs). The 3' end was similar to the other scFv VH molecules with an out of frame BclI site fused to the VTVSS type sequence at the end of the VH domain.

PCR oligos:

40.2.220 scFv:

5' primer--40.2.220S5: 5'-gttgtgtcgcacattgttctgactcagctccagccaccctgtc-3' (SEQ ID NO)

3' primer--40.2.220Bcl3: 5'-gttgttgatcagagacagtgtccctgg-3' (SEQ ID NO)

10 Linker Primers:

BclI-SalI fragment created by annealing complementary oligonucleotides. This fragment is then ligated into BclI digested vector along with a SalI-BclI scFv to create the (linker-scFv) BclI fragment desired for shuttling.

5' primer: 5'-

15 gatcaatccaactctgaagaagcaaagaagaggaggccaaaaaggaggaagccaagaaatctaacagcg-3' (SEQ ID NO)

3' primer: 5'-tcgacgtgttagatttcttgcttctctcttttggcctcccttcttcttgccttctcagagttggatt-3' (SEQ ID NO)

20 This BclI fragment was then ligated downstream of the 2H7 scFv in pD18-Ig. Transformants were screened for the presence of a 2.4 kb HindIII-Xba insert and positive clones sequenced prior to further studies. COS cell transient transfections were performed with this construct and culture supernatants screened for the presence of protein of the predicted size and for binding to CD20 and to CD40 transfected CHO cells.

25 New bispecific constructs can be created by designing hinge type linkers which incorporate one or preferably two restriction sites at either end of the linker, facilitating asymmetric digests and transfer of (linker-scFv) or (scFv-linker) cassettes between different constructs. These constructs will also incorporate the VH L11S and other V region substitutions, which presumably facilitate proper folding and result in increased expression of the molecules in which they are inserted.

30 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2

same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

5

EXAMPLE 153**2H7 scFv IgAH IgACH2 T4CH3-hCD80 TM/CT**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113 and 119. This construct has previously been referred to as 2H7 scFv, IgA hinge IgA-T4-CD80 and 2H7 scFv IgAH IgA-T4-CD80, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

10
15**EXAMPLE 154****G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD80 TM/CT**

This construct has a G19 (anti-CD3) single chain Fv binding region described in Example 29. This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

20
25**EXAMPLE 155****2E12 scFv (CCC-P) WH WCH2 WCH3-hCD80 TM/CT**

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1. This connecting region is attached

30

to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

5

EXAMPLE 156**2H7 VHL11S scFv (SSS-S) IgECH3CH4**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine. as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to human IgE CH3 and CH4 constant region. This truncated constant region was created according to the methods described in Example 38. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

10

15

EXAMPLE 157**IGD HINGE**

An alternative hinge region can be isolated from human IgD immunoglobulin hinge region by using PCR assay to isolate the desired region. The PCR reaction is the same used in Example 1. This hinge was truncated by 6 amino acid residues at the 3' end. The primers used in this PCR reaction are listed below.

20

5'' Primer: 5'-GTGGATCCAGGTTCGAAGTCTCCAAAGGCACAGGCC-3' (SEQ ID NO)

25

3' primer: 5'-GTTGTCGACTGCACCGGTCTTTGTCTCTCTCTCTTC-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 158**hCD28 TM/CT**

30

For some of the cell surface ORF constructs, the transmembrane domain of CD80 was substituted with the transmembrane domain of human CD28 because it forms a

dimer on the cell surface rather than a monomer as the CD80 does. Several of the molecules which drive the apoptotic program require oligomerization/trimerization to form a signaling complex; therefore, it is important to be able to control initiation of signaling by controlling the degree of oligomerization of these recombinant receptors on the cell surface.

The primers used in PCR amplification of the CD28 tail are given below:

5' Primer: 5'-gttgtggatccttcgaacccctttgggtgctggtggtggtgga-3' (SEQ ID NO)

3' primer: 5'-gttgttatcgatctcgagtcaggagcgataggctgcgaagtc-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 159

2H7 scFv VH L11S (SSS-S) H K322L CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322L mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a leucine using overlapping PCR described in Example 56, but with different primers for the first PCR reaction, which are listed below.

5' primer: 5'ttcctctccccccaaacccaaggacaccctcatgatctcccggaaccctgaggtcac-3' (SEQ ID NO)

3' primer: 5'-ggacagtgggagtgacc-3' (SEQ ID NO)

PCR product was cloned into TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 160

2H7 scFv VH L11S (CSS-S) H K322L CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the

leucine has been changed to a serine, as described in Example 33. This binding region is attached to a mutant IgG connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322L mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a leucine using overlapping PCR described in Example 56, using primers from Example 159 in the first PCR reaction and primers from Example 57 for the second PCR reaction.

PCR products were cloned into the TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO:__.

EXAMPLE 161

IN VIVO B CELL DEPLETION BY 2H7 scFv (SSS-S) H WCH2 WCH3 AND 2H7 scFv (SSS-S) H P238SCH2 WCH3

This Example describes experiments relating to ADCC effector mechanisms in the depletion of B cells through comparison of the activities of an anti-CD20 construct (2H7 scFv (SSS-S) H WCH2 WCH3) and a anti-CD20 construct with a proline to serine amino acid substitution in the CH2 domain (2H7 scFv (SSS-S) H P238SCH2 WCH3). Figure 72 shows the results of an experiment on apoptosis induction by these constructs. The results indicated that both molecules bind CD20 and induce apoptosis that is mediated by activation of caspase 3.

Figure 73 illustrates that anti-CD20 constructs mediate CDC activity towards CD20 positive target cells. A 2H7 scFv (SSS-S) H WCH2 WCH3 construct, a 2H7 scFv (SSS-S) H P238SCH2 WCH3 construct, or Rituximab were incubated at increasing concentrations with 10^4 Bjab Target Cells and a 1:10 dilution of rabbit complement (PelFreez) in a volume of 100 microliters for sixty minutes. Aliquots were stained with trypan blue (Invitrogen), and counted using a hemacytometer to determine the percentage of the cell population killed during treatment. Negative controls with cells and only one reagent were also included. Figure 74 illustrates that the 2H7 scFv (SSS-S) H WCH2 WCH3 construct was effective in ADCC with peripheral blood mononuclear cells. ADCC activity of 2H7 scFv (SSS-S) H WCH2 WCH3 or Rituximab was measured *in vitro* against BJAB B

lymphoma cell line as the target cells, and using fresh human PBMC as effector cells. Effector to target ratios were varied as follows: 100:1, 50:1, and 25:1, with the number of BJAB cells per well remaining constant but varying the number of PBMC. BJAB cells were labeled for 2 hours with ^{51}Cr and aliquoted at a cell density of 5×10^4 cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or Rituximab were added at a concentration of 10 $\mu\text{g/ml}$, and PBMC were added at 1.25×10^6 cells/well (25:1), 2.5×10^6 cells/well (50:1), or 5×10^6 cells/well (100:1), in a final volume of 200 μl . Natural killing was measured at each effector:target ratio by omission of the construct or MAb. Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 5 hours, and 100 μl culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter. Figure 75 illustrates that the 2H7 scFv (SSS-S) H WCH2 WCH3 construct bound soluble CD16 fusion protein (constructed from either high or low affinity alleles (158V/F)), while the 2H7 scFv (SSS-S) H P238SCH2 WCH3 construct did not show detectable binding. CD20 CHO cells (10^6) were incubated with saturating amounts of 2H7 scFv (SSS-S) H WCH2 WCH3 or 2H7 scFv (SSS-S) H P238SCH2 WCH3 (10 $\mu\text{g/ml}$) for one hour on ice in PBS/2% FBS. Cells were washed in PBS/2% FBS and incubated with serial dilutions of 0.5 mg/ml FITC-CD16 for one hour on ice. Cells were washed and specific binding measured by flow cytometry using a Beckman-Coulter Epics C machine. Results were analyzed using Expo analysis software and normalized fluorescence units graphed as a function of concentration. Figure 76 illustrates an experiment on the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238SCH2 WCH3 constructs to bind U937 cells. U937 cells (10^6) expressing CD64 were incubated in PBS/2%FBS for one hour on ice with 2H7 scFv (SSS-S) H WCH2 WCH3 or 2H7 scFv (SSS-S) H P238SCH2 WCH3. Cells were washed and incubated for one hour on ice with FITC-goat anti-human IgG1 (Fc specific) (Caltag) at a final dilution of 1:100. Cells were washed and fluorescence analyzed on a Beckman-Coulter EpicsC flow cytometer. Data was analyzed using Expo analysis software, and fluorescence intensity graphed as a function of the concentration of 2H7 scFv (SSS-S) H WCH2 WCH3 or 2H7 scFv (SSS-S) H P238SCH2 WCH3. This figure shows that both the 2H7 scFv (SSS-S) H WCH2 WCH3 construct and

the 2H7 scFv (SSS-S) H P238SCH2 WCH3 construct bound to U937 cells with equivalent titration curves. The results indicated that the 2H7 scFv (SSS-S) H P238SCH2 WCH3 construct was not impaired in its binding to the high affinity Fc receptor FcγRI (CD64). The fact that binding to high affinity FcγRI on U937 cells was similar for the two molecules in this experiment indicated that the P238S amino acid change selectively reduced the binding to FcγRIII. Figure 77 illustrates B cell depletion in macaques mediated by a anti-CD20 construct (2H7 scFv (SSS-S) H WCH2 WCH3) and a anti-CD20) scFv with a CH2 domain mutation. 2H7 scFv (SSS-S) H WCH2 WCH3 or 2H7 scFv (SSS-S) H P238SCH2 WCH3 were administered to macaques (*M. fascicularis*) by intravenous injection at 6 mg/kg, with two infusions given one week apart. The effect on circulating B cells was measured by detection of CD40 positive B cells in peripheral blood. Blood samples were drawn from injected animals at days 7,0,1,3,7,8,10,14, 28, and 43. B cell depletion was estimated by performing CBC (complete blood counts) and two-color flow cytometry analysis on monkey blood. FITC or PE conjugates of antibodies against CD40, CD19, CD20, IgG, CD3, CD8 were used in various combinations. Data are plotted as the number of CD40 positive blood B cells tabulated in thousands of cells per microliter over time relative to the initial pre-injection time point level of B cells (maximum). This experiment showed that 2H7 scFv (SSS-S) H WCH2 WCH3 scFv injection resulted in rapid and complete depletion of circulating B cells that lasted longer than 28 days following the second injection. Injection of 2H7 scFv (SSS-S) H P238SCH2 WCH3 scFv did not completely deplete B cells even though CD20 epitopes were saturated. A slow reduction in B cells to approximately 50% of initial levels was observed during the first 2 weeks, but B cells rapidly returned to starting levels in these animals. These results indicate that the ability to bind high affinity FcγRI and to mediate complement dependent cytotoxicity is not sufficient for a CytosB20G molecule to completely deplete circulating B cells, and that ADCC mediated by interaction with CD16 is likely necessary for rapid and sustained B cell depletion.

These experiments indicated that the 2H7 scFv (SSS-S) H WCH2 WCH3 scFvs are able to trigger B cell depletion functions through three different mechanisms of action (1) induction of apoptosis, (2) CDC effector mechanisms, and (3) ADCC effector mechanisms. All three of these mechanisms probably contribute to depletion of B cells *in vivo*. The data indicate that complete and sustained depletion of B cells requires intact ADCC effector

mechanisms. However, the partial depletion obtained with an ADCC negative mutant also indicates that apoptosis and CDC mechanisms contribute to mediating a portion of the total B cell depletion effects. The results support the use of 2H7 scFv (SSS-S) H WCH2 WCH3 scFv in patients with a CD20 positive B cell malignancy or autoimmune disease.

5

EXAMPLE 162

INDUCTION OF APOPTOSIS IN B CELLS BY ANTI-CD37 GS-1 VL11S scFv (SSS) H
WCH2WCH3 CONSTRUCTS

In this Example, anti-CD37 GS-1 VL11S scFv (SSS) H WCH2WCH3 constructs were evaluated for their ability to bind target cells and for their ability to induce apoptosis.

10 The constructs were also physically characterized by size-exclusion chromatography (SEC). Figure 78 illustrates the SEC profile of G28-1 (anti-CD37) constructs having SSC hinge domain forms (GS-1 VL11S scFv (SSS) H WCH2WCH3). G28-1 (anti-CD37) constructs were purified from CHO culture supernatants by Protein A affinity chromatography. Purified aliquots of 10-25 mg were subjected to HPLC over a Tosoh

15 Biosep, Inc. TSK 3000 SWXL HPLC column, pore size 5 mm. The flow rate was 1ml/min, in PBS, pH 7.2 running buffer. Migration rates of molecular weight standards are indicated below the tracing. The GS-1 VL11S scFv (SSS) H WCH2WCH3 construct is indicated in blue, while the GS-1 VL11S scFv (SSS) H WCH2WCH3 construct is indicated in red. The GS-1 VL11S scFv (SSS) H WCH2WCH3 construct generated a

20 uniform peak of approximately 75-100 kDa, while the GS-1 VL11S scFv (SSS) H WCH2WCH3 construct generated a smaller form and other heterogeneous forms, including a high molecular weight form greater than 200 kDa. Figure 79 illustrates the binding of G28-1 (anti-CD37) constructs to B cell lymphoma cell lines. Serial dilutions of purified GS-1 VL11S scFv (SSS) H WCH2WCH3 or GS-1 VL11S scFv (SSS) H

25 WCH2WCH3 were incubated with 10^6 cells of each cell type for 60 minutes on ice in PBS/2%FBS. Samples were washed twice, and incubated with a mixture of FITC goat anti-human IgG and FITC goat anti-human IgG F(ab')₂ (CalTag) at 1:100 each, on ice for 45 minutes. Samples were washed and analyzed by flow cytometry using a FACScalibur (Becton-Dickinson). The results of this experiment show that both GS-1 VL11S scFv

30 (SSS) H WCH2WCH3 and GS-1 VL11S scFv (SSS) H WCH2WCH3 constructs bound to BJAB and Ramos cells, and moderately to WIL-2 cells. G28-1 (anti-CD37) (SSS)H G scFv or G28-1 (SSS)H G scFv constructs molecules bound Raji and Namalwa cells at

lower levels in these experiments. Binding to all lines was significant because background fluorescence has been subtracted from the data shown in Figure 79. Figure 80 illustrates the binding of annexin and v-propidium iodide to Ramos cells after overnight incubation with GS-1 VL11S scFv (SSS) H WCH2WCH3 or GS-1 VL11S scFv (SSC) H WCH2WCH3 forms of scFvs. AnnexinV-PI staining of Ramos Cells incubated for 24 hours with G28-1 (anti-CD37) scFvs. Ramos B cells at 10^6 cells/ml were incubated in 12 well dishes for 24 hours with G28-1 (anti-CD37) scFvs at 10 mg/ml, in a total volume of 2 ml. Cells were stained with annexinV and propidium iodide with a kit obtained from Immunotech, according to manufacturer's instructions. Samples were analyzed by two-color flow cytometry using a FACsCalibur flow cytometer (Becton-Dickinson). The % of the total cells in each quadrant is indicated next to each dot plot. Figure 83 shows the binding of Annexin V-Propidium Iodide to cells after overnight incubation with GS-1 VL11S scFv (SSS) H WCH2WCH3 or GS-1 VL11S scFv (SSC) H WCH2WCH3. The results indicated that both GS-1 VL11S scFv (SSS) H WCH2WCH3 and GS-1 VL11S scFv (SSC) H WCH2WCH3 constructs were able to induce apoptosis, but that the SSC form induced more apoptosis than the SSS form. Figure 81 illustrates the inhibition of proliferation of Ramos cells grown in the presence of G28-1 (anti-CD37) constructs. Ramos B cells were incubated with serial dilutions of purified G28-1 (anti-CD37) constructs containing either the IgG1 hinge identified as (SSS)H or (SSC)H. Cultures were incubated in 96 well flat bottom tissue culture dishes (Costar) at 37°C, 5%CO₂ for 36 hours prior to pulsing with ³H-thymidine for the last 12 hours of a 48 hour incubation (0.75 mCi/well). Cells were harvested onto 96-well GFC plates using a Packard harvester, dried, and 25 ml Microscint scintillation fluid added to each well prior to counting on a TopCount NXT microplate (Packard) scintillation counter. Data are plotted as cpm incorporated versus protein concentration. Each construct showed increasing inhibition of proliferation with increasing protein concentration. Figure 82 illustrates the induction of apoptosis in Ramos B cells cultured in the presence of and 2H7 (anti-CD20) and G28-1 (anti-CD37) constructs. Ramos B cells were incubated with CD20 and/or CD37 targeted constructs (10 mg/ml) in solution for 20 hours. The cells were then harvested, washed, and incubated in annexinV and propidium iodide using a staining kit from Immunotech prior to two color flow cytometry using a FACsCalibur flow cytometer (Becton-Dickinson). The graph shows the percentage of annexin V positive cells identified by their staining in the

right quadrants of the dot plots. The results of this experiment indicate that the both G28-1 constructs were more efficient than the 2H7 construct and that the G28-1 VL11S scFv (SSS) H WCH2 WCH3 construct was more efficient than the G28-1 VL11S scFv (SSC) H WCH2 WCH3 construct. However, the amount of apoptosis of Ramos cells was greatest when the 2H7 and the G28-1 constructs were used in combination. Figure 83 illustrates the killing of Ramos cells by complement dependent cytotoxicity (CDC) induced by 2H7 (anti-CD20) constructs and G28-1 (anti-CD37) constructs with hinge region mutations. 2H7 scFv (CSS-S) H WCH2 WCH3 (anti-CD20), G28-1 scFv (SSS)H G, G28-1 (SCS)H (anti-CD37-scFv), G28-1 (CSS)H (anti-CD37-scFv), or G28-1 (SSC)H (anti-CD37-scFv) were incubated at 10 mg/ml with 10⁴ Ramos Target Cells and a 1:10 dilution of rabbit complement (PelFreez) in a volume of 150 ml for 90 minutes. Aliquots were stained with trypan blue (Invitrogen), and counted using a hemacytometer to determine the percentage of the cell population killed during treatment. Negative controls with cells and only one reagent were also included. Both G28-1 (SSS) (anti-CD37 scFv) and G28-1 (SSC) (anti-CD37 scFv) rapidly killed Ramos cells in the presence of rabbit complement in this experiment. The activity of G28-1 (SSC) (anti-CD37 scFv) was higher than that of G28-1 (SSS) (anti-CD37 scFv), and was nearly as potent in this assay as the CD20-directed 2H7 scFv (CSS-S) H WCH2 WCH3 scFv molecule. Figure 84 illustrates the induction of ADCC of Ramos cells incubated with G28-1 (anti-CD37) scFvs. G28-1 (anti-CD37) constructs at 10 mg/ml were incubated in flat-bottom 96 well plates with 10⁴ ⁵¹Cr-labeled Ramos cells and resting human PBMCs at different effector:target ratios ranging from 0 to 100. All incubations were performed in triplicate at each effector:target ratio. Natural killing was measured at each effector:target ratio by omission of the constructs. Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 6 hours, and 100 ml culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter. Results from these experiments indicated that the G28-1 (anti-CD37) constructs bind to target cells, that they induce apoptosis in the B cell lines, and that they mediate effector functions including CDC and ADCC.

Additional non-limiting representative constructs or sequences within, or useful within, the present inventions are as follows:

HuIgG1 wild type hinge, CH2, CH3 (nucleotide sequence) (SEQ ID NO: __)

5 tctgatcaggagcccaaattctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagtctt
cctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgctgtggtggacgtgagccacgaa
gaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagcagtacaa
cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaac
aaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccc
10 atcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaaggcttctatccagcgacatcgccgtggagtgg
gagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctcctgtctccgggtaaatgatctaga

15 **HuIgG1 wild type hinge, CH2, CH3** (amino acid sequence) (SEQ ID NO: __)

SDQEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSMHEALH
20 NHYTQKSLSLSPGK

Llama IgG1 hinge, CH2, CH3 (nucleotide sequence) (SEQ ID NO: __)

tgatcaagaaccacatggaggatgcacgtgccncagtgccncnaatgccngcncngaaactnccaggaggcccttctgtcttt
gtcttcccccgaaacccaaggacgtcctctccatttttgaggccgagtcacgtgcgtttagtggtgacgtcggaagaaagaccc
25 cgaggtcaatttcaactggtatattgatggcgttgaggtgcgaacggccaatacgaagccaaaagaggaacagttcaacagcacg
taccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaaggaattcaagtgaaggtcaacaacaaagct
ctccggcccccatcgagaggaccatctccaaggccaaagggcagaccgggagccgaggtgtacaccctggccccacacc
gggaagaactggccaaggacaccgtgagcgtaacatgcctggtcaaaggcttctaccagctgacatcaacgttgagtggcaga
ggaacggtcagccggagtcagagggcacctacccaacacgccgccacagctggacaacgacgggacctacttctctacagc
30 aagctctcggtgggaaagaacacgtggcagcggggagaaaccttaacctgtgtggtgatgcatgaggccctgcacaaccactac
accagaaatccatcaccagttctcgggtaaatagtaatactaga

Llama IgG1 hinge, CH2, CH3 (in figure 23 as Llama IgG1) (amino acid sequence)
(SEQ ID NO:___)

EPHGGCTCPQCPAPELPGGPSVFVFPPKPKDVLSISGRPEVTCVVVDVGKEDPEVN
5 FNWYIDGVEVRTANTKPKKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKAL
PAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRN
GQPESEGTYANTPPQLDNDGTYFLYSRLSVGKNTWQRGETLTGVVMHEALHNHY
TQKSITQSSGK

10 **Llama IgG2 (nucleotide sequence) (SEQ ID NO:___)**

tgatcaagaacccaagacacacaaaaccacaaccacaaccacaacccaatcctacaacagaatccaagtgtcccaaatgt
ccagcccctgagctcctgggagggccctcagttctctctcccccgaaacccaaggacgtcctctccatttctgggagggccga
ggtcacgtgcgttggttagacgtgggccaggaagaccccgaggtcagttcaactggtacattgatggcgctgaggtgcgaacg
gccaacacgaggccaaaagaggaacagttaacagcacgtaccgcgtggtcagcgtcctgccccatccagcaccaggactggct
15 gacggggaaggaattcaagtcaaggtcaacaacaaagctctccggccccatcgagaagaccatctccaaggccaaagggc
agaccggggagccgcaggtgtacacctggccccacaccgggaagagctggccaaggacaccgtgagcgtaacatgcctggt
caaaggcttctaccacctgatataacgtgagtgagcagaggaatgggcagccggagtcagagggcacytacgccaccacgc
caccacagctggacaacgacgggacctacttctctacagcaagctctcgggtgggaaagaacacgtggcagcagggagaaacc
ttcacctgtgtggtgatgcacgaggccctgcacaaccactacaccagaaatccatcaccagctctcgggtaaataagtaattaga.

20

Llama IgG2 (amino acid sequence) (SEQ ID NO:___)

DQEPKTPKPQPQPQPQNPPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLSISGRPEV
TCVVVDVGQEDPEVSFNWYIDGAEVRTANTRPKKEEQFNSTYRVVSVLPIQHQDWL
TGKEFKCKVNNKALPAPIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVK
25 GFYPPDINVEWQRNGQPESEGTYATTPPQLDNDGTYFLYSKLSVGKNTWQQGETF
TCVVMHEALHNHYTQKSITQSSGK

Llama IgG3 Fc (nucleotide sequence) (SEQ ID NO:___)

tgatcaagcgcaccacagcgaagacccagctccaagtgtcccaatgcccaggccctgaactccttgaggggccacggtctt
30 catcttcccccgaaagccaaggacgtcctctccatcacccgaaaacctgaggtcacgtgcttggtggacgtgggtaaagaag
acctgagatcgagttcaagctggtccgtggatgacacagaggtacacaggctgagacaaagccaaaggaggaacagttaac

agcacgtaccgcgtggcagcgtcctgccatccagcaccaggactggctgacggggaaggaattcaagtgaaggtcaacaa
 caaagctctcccagccccatcgagaggaccatctccaaggccaaagggcagaccgggagccgcaggtgtacacctggcc
 ccacaccgggaagagctggccaaggacaccgtgagcgtaacctgcctggctaaaggcttctccagctgacatcaacgttgagt
 ggcagaggaatgggcagccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttct
 5 ctacagcaaactctccgtgggaaagaacacgtggcagcagggagaagtcttcacctgtgtggtgatgcagaggctctacacaat
 cactccaccagaaatccatcacccagtcttcgggtaaataagtaatctagaggggccc

Llama IgG3 Fc (amino acid sequence) (SEQ ID NO:___)

DQAHHSSEDPSSKCPKCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKK
 10 TLRSSSSWSVDDTEVHTAETKPKKEEQFNSTYRVVSVLP IQHQDWLTGKEFKCKVN
 NKALPAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVE
 WQRNGQPESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEA
 LHNHSTQKSITQSSGK

HuIgG1 wild type hinge (nucleotide sequence) (SEQ ID NO:___)

gatcaggagcccaaatcttgacaaaaactcacacatgccaccgtgccagca

HuIgG1 wild type hinge (amino acid sequence) (SEQ ID NO:___)

DQEPKSCDKTHTCPPCPA

20

HuIgG1 H2, wild type hinge with leu at second position (nucleotide sequence) (SEQ ID NO:___)

gatctggagcccaaatcttgacaaaaactcacacatgccaccgtgccagca

HuIgG1 H2, wild type hinge with leu at second position (amino acid sequence) (SEQ ID NO:___)

DLEPKSCDKTHTCPPCPA

NTHuIgG1 wild type CH2 (nucleotide sequence) (SEQ ID NO:___)

cctgaactcctgggggggaccgtcagttcttcttcccccaaaaccaaggacaccctcatgatctcccggaccctgaggtcac
 atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
 gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
 5 gcaaggagtacaagtgaaggtctccaacaaagccctcccagcccccctcgagaaaaccatctccaaagccaaa

HuIgG1 wild type CH2(amino acid sequence) (SEQ ID NO:___)

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

10

HuIgG1 wild type CH3 (nucleotide sequence) (SEQ ID NO:___)

gggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
 ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
 ccgtgctggactccgacggctccttcttctctatagaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctca
 15 tgctccgtgatgcattgaggtctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgG1 wild type CH3 (amino acid sequence) (SEQ ID NO:___)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP
 VLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

20

HuIgG1 mutated hinge (C-C-C→S-S-S) (nucleotide sequence) (SEQ ID NO:___)

gatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagca

HuIgG1 mutated hinge (C-C-C→S-S-S) (amino acid sequence) (SEQ ID NO:___)

25 DQEPKSSDKTHTSPSPA

HIgG1MTH WTCH2CH3 (mutant hinge with wild type CH2 and CH3 (reads from the hinge+Ig tail) (nucleotide sequence) (SEQ ID NO:___)

tgatcaccccaaatcttctgacaaaactcacacatctccaccgtcctcagcacctgaactcctgggtggaccgtcagtcttctcttcc
 ccccaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtgggtgggtggacgtgagccacgaagaccctg
 aggtcaagttcaactggtagctggacggcgtggaggtgcataatgccaaagacaaagccgaggagcagtagacaacagcacg
 taccgtgtggtagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaagggtctccaacaagccc
 5 tcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatccggg
 gatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagc
 aatgggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctcaccg
 tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
 gcctctccctgtctccgggtaaatgataatctaga

10

Mutant hinge, but wild type CH2 and CH3 (amino acid sequence) (SEQ ID NO: __)

DHPKSSDKTHTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
 EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
 NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEV
 15 ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNH
 YTQKSLSLSPGK

2H7 scFv llama IgG1 (nucleotide sequence) (SEQ ID NO: __)

aagcttgcgccatggattttcaagtgcagatttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctt
 20 ccagltccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcact
 ggtaccagcagaagccagatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagt
 gcagtgggtctgggaccttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggatt
 taaccacccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtgggtgagcttgaggaggtg
 ggagctctcaggcttatctacagcagcttggggctgagctggtagggcctggggcctcagtgaagatgtcctgcaaggcttctggc
 25 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaactcttactgggtacttcgatgtctggggcac
 agggaccacgggtcaccgtcttctgatcaagaaccacatggaggatgcacgtgccncagtgccncaatgccngcncng
 aactnccaggaggcccttctgtcttcttcccccgaaaccaaggacgtcctctccattttggaggccgagtcacgtgcgttgt
 30 agtggagctcggaaagaaagacccccgaggtcaatttcaactgggtatattgatggcgttgaggtgcgaacggccaatacgaagcca
 aaagaggaacagttcaacagcacgtaccgcgtgggtcagcgtcctgcccattccagcaccaggactgggtgacggggaaggaatt

caagtgcagggtcaacaacaaagctctcccgcccccatcgagaggaccatctccaaggccaaagggcagacccgggagccg
 caggtgtacacctggccccacaccgggaagaactggccaaggacaccgtgagcgtaacatgcctggtaaaaggcttctacca
 gctgacatcaacgttgagtgagcaggaacgggtcagccggagtcagagggcacctacgccaacacgccgccacagctggaca
 acgacgggacctaacttctctacagcaagctctcgggtgggaaagaacacgtggcagcggggagaaaccttaacctgtgtggtgat
 5 gcatgaggccctgcacaaccactacacccagaaatccatcacccagttctcgggtaaatagtaatctaga

2H7 scFv llama IgG1 (amino acid sequence) (SEQ ID NO:)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSPGGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 10 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPHGGCT
 CPQCPAPELPGGPSVFVFPKPKDVLISFGGRVTCVVVDVGKKDPEVNFNHWYIDGV
 EVRTANTKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPAPIERTISK
 15 AKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRNGQPESEGT
 ANTPQLDNDGTYFLYSKLSVGKNTWQRGETLTCVVMHEALHNHYTQKSITQSS
 GK

2H7 scFv llama IgG2 (nucleotide sequence) (SEQ ID NO:)

aagcttgcgcgcatggatttcaagtgcagatttcagcttctgtaatacgtgcttcagtcataattgccagaggacaaattgttctct
 cccagctccagcaatcctgtctgcattctccaggggagaagggtcacaatgacttcagggccagctcaagtgttaagttacatgcact
 ggtaccagcagaagccaggatcctccccaaacctggattatgccccatccaacctggcttctggagtcctgctcgttcagtg
 gcagtggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggattt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtg
 25 ggagctctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgagatgtcctgcaaggcttctggc
 tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaat
 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
 agggaccacggtcaccgtcttctgatcaagaacccaagacacaaaaccacaaccacaaccacaacccaatcctac
 30 aacagaatccaagtgtccaaatgtccagcccctgagctcctgggagggccctcagttctatcttcccccgaaacccaaggac
 gtcctctccatttctgggaggcccaggtcacgtgcgtgtgtgtagacgtgggccaggaagacccccgaggtcagttcaactggta

cattgatggcgctgaggtgcgaacggccaacacgaggccaaaagaggaacagtcaacagcagctaccgcgtgggtcagcgtcc
 tgcccatccagcaccaggactgggtgacggggaaggaattcaagtcaaggtcaacaacaagctctccggcccccatcgag
 aagaccatctccaaggccaaagggcagacccgggagccgaggtgtacaccctggccccacaccgggaagagctggccaag
 gacaccgtgagcgtaacatgcctgggtcaaaggcttctaccacctgatatcaacgttgagtggcagaggaatgggcagccggagt
 5 cagaggggcacytacgccaccacgccaccccagctggacaacgacgggacctacttctctacagcaagctctcggtgggaaag
 aacacgtggcagcagggagaaaccttcacctgtgtgggtgatgcacgaggccctgcacaaccactacaccagaaatccatcacc
 cagttctcgggtaaatagtaatctaga

AA2H7 scFv llama IgG2 (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 10 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKTPKP
 QPQPQPQNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLISGRPEVTCVVVDVG
 15 QEDPEVSFNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCK
 VNNKALPAPIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPPDIN
 VEWQRNGQPESEGTYATTPQLDNDGTYFLYSKLSVGKNTWQQGETFTCVVMHE
 ALHNHYTQKSITQSSGK

NT2H7 scFv llama IgG3 (nucleotide sequence) (SEQ ID NO: __)

aagcttcccgcctggatttcaagtgcagatttccagcttctgctaatacagtccttcagtcataattgccagaggacaaattgttctct
 20 cccagctccagcaatctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcact
 ggtaccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtg
 gcagtggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggattt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
 25 ggagctctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggc
 tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggcttatttctgtgcaagagtgggtgactatagtaacttactgttacttcgatgtctggggcac
 agggaccacggtcaccgtctcttctgatcaagcgaaccacagcgaagaccccagctccaagtgtcccaaatgccaggccctga
 30 actccttggagggcccacggtcttcttctcccccgaaagccaaggacgtcctctccatcaccggaaaacctgaggtcacgtgctt
 gtggtggacgtgggtaaagaagaccctgagatcgagttcaagctggctccgtggatgacacaggtacacacggctgagacaaa

gccaaaggaggaacagttaacagcacgtaccgctgggtcagcgtcctgccatccagcaccaggactgggtgacggggaag
 gaattcaagtgaaggtcaacaacaaagctctccagccccatcgagaggaccatctccaaggccaaagggcagacccggga
 gccgcaggtgtacacctggccccacaccgggaagagctggccaaggacaccgtgagcgtaacctgcctgggtcaaaggcttct
 cccagctgacatcaacgttgagtggcagaggaatgggcagccggagtcagagggcacctacccaacacgccgccacagctg
 5 gacaacgacgggacctacttctctacagcaactctccgtgggaaagaacacgtggcagcagggagaagtcttcacctgtgtg
 tgatgcacgaggctctacacaatcactccaccagaaatccatcaccagctcttcgggtaaatagtaatctagagggccc

2H7 scFv llama IgG3 (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 10 QQKPGSSPKPWTYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQAHSHSED
 PSSKCPKCPGPELLGGPTVFIFPPKAKDVLISITRKPEVTCLWWTWVKKTLRSSSSW
 15 SVDDTEVHTAETKPKKEEQFNSTYRVVSVLPQHQDWLTGKEFKCKVNNKALPAPI
 ERTISKAKGQTREPQVYTLAPHREELAKDTSVVTCLVKGFFPADINVEWQRNGQP
 ESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHSTQK
 SITQSSGK

20 2H7 scFv WTH WTCH2CH3 (nucleotide sequence)

aagcttcccgcctggaatttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 cccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgaagtacatgcact
 ggtaccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtg
 gcagtggtgtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagttt
 25 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggc
 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaacttactggtacttcgatgtctggggcac
 30 agggaccacgggtcaccgtctcttctgatcaggagcccaaatcttgtacaaaactcacacatgccaccgtgccagcacctgaac
 tcctggggggaccgtcagcttctcttcccccaaaaccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtg

gtggtggacgtgagccacgaagaccctgagggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
 ccgcgaggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaagga
 gtacaagtgaaggtctccaacaaagccctccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaac
 cacaggtgtacaccctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatcc
 5 cagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccggtgctggactccgac
 ggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
 ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv WTH WTCH2CH3 (amino acid sequence) (SEQ ID NO:___)

10 MDFQVQIFSFLNISASVILARGQIVLSQSPAILSPGGEKVTMTCRASSSVSYMHWY
 QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYNSNSYWFYFDVWGTGTTVTVSSDQEPKSCDK
 15 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSL
 SPGK

20 **NTCD80 transmembrane domain and cytoplasmic tail (+restriction sites) (nucleotide sequence) (SEQ ID NO:___)**

gaggatccttcgaacctgctccatcctggccattaccttaatctcagtaaattgaattttgtgatatgctgcctgacctactgcttg
 cccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgat

25 **CD80 transmembrane domain and cytoplasmic tail (amino acid sequence) (SEQ ID NO:___)**

ADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

40.2.220 VL (anti-human CD40 scFv #1-VL) (nucleotide sequence) (SEQ ID NO:___)

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgttctgactc
 agtctccagccaccctgtctgtgactccaggagatagagtctcttcttctgcagggccagccagagtattagcgactacttacactg
 gtatcaacaaaaatcacatgagtcctcaaggcttctcatcaaatatgcttccattccatctctgggatcccctccaggttcagtgga
 gtggatcagggtcagatttcactctcagtatcaacagtggtgaacctgaagatgttgaatttattactgtcaacatggtcacagctttc
 5 cgtggacgttcggtggaggcaccaagctggaaatcaaacgg

40.2.220 VL (anti-human CD40 scFv #1--VL) (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
 YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLINSVEPEDVGIYYCQHGHSF
 10 PWTFGGGTKLEIKR

40.2.220 VH (for anti-human CD40 scFv #1--VH) (nucleotide sequence) (SEQ ID NO: __)

cagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctctgcaaggcttctgggtatgcctt
 15 cacaactactggaatgcagtggtgcaagagatgccaggaaagggttgaagtggattggtggataaacaccccactctggagt
 gccaaaatatgtagaagacttcaaggacggttgccttctcttggaaacctctgccaaactgcataattacagataagcaacctcaa
 agatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatgacctggcctacttgccttactggggccaaggac
 actggtcactgtctctgatca

40.2.220 VH (for anti-human CD40 scFv #1--VH) (amino acid sequence) (SEQ ID NO: __)

QIQLVQSGPELKKPGETVRISCKASGYAFTTTGMQWVQEMPGKGLKWIGWINTPL
 WSAKICRRLQGRFAFSLETSANTAYLQISNLKDEDTATYFCVRSNGNGNYDLAYFA
 YWGQGTLVTVS

25

40.2.220 scFv (anti-human CD40 scFv #1) (nucleotide sequence) (SEQ ID NO: __)

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgttctgactc
 agtctccagccaccctgtctgtgactccaggagatagagtctcttcttctgcagggccagccagagtattagcgactacttacactg
 gtatcaacaaaaatcacatgagtcctcaaggcttctcatcaaatatgcttccattccatctctgggatcccctccaggttcagtgga
 30 gtggatcagggtcagatttcactctcagtatcaacagtggtgaacctgaagatgttgaatttattactgtcaacatggtcacagctttc

cgtggacgttcggtggaggcaccaagctggaatcaaacggggtggcgggtggctcggcgagggtgggtcgggtggcggcg
 gatctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtat
 gccttcacaactactggaatgcagtgggtgcaagagatgccaggaaagggttggaaggattggctggataaacaccccactctg
 gagtgcacaaaatatgtagaagacttcaaggacggttgcttctctttgaaacctctgccaactgcataattacagataagcaacc
 5 tcaaagatgaggacacggctacgtattctgtgtgagatccgggaatggttaactatgacctggcctactttgcttactggggccaag
 ggacactggtcactgtctctgatca

40.2.220 scFv (anti-human CD40 scFv #1) (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
 10 YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSF
 PWTFGGGTKLEIKRGGGGSGGGSGGGSGSIQLVQSGPELKKPGETVRISCKASG
 YAFTTTGMQWVQEMPQGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAY
 LQISNLKDEDTATYFCVRSGNGNYDLAYFAYWGQGLVTVS

15 2e12 VL (with L6 VK leader peptide) (nucleotide sequence) (SEQ ID NO: __)

atggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatigtgctcacccaalctc
 cagcttctttggctgtgtctctaggtcagagagccaccatctctgcagagccagtgaaagtgttgatattatgtcacaagtttaatgc
 agtggtagcaacagaaaccaggacagccacccaaactcctcatctctgctgcaccaacgtagaatctggggtccctgccaggttt
 agtggcagtggggtctgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtatttctgtcagcaaaagta
 20 ggaagggtccttgacgttcggtggaggcaccaagctggaatcaaacgg

2e12 VL (with L6 VK leader peptide) (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPLKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
 25 CQQSRKVPWTFGGGTKLEIKR

2e12 VH (no leader peptide) (nucleotide sequence) (SEQ ID NO: __)

caggtgcagctgaaggagtcaggacctggcctggcggcgcctcacagagcctgtccatcacatgcaccgtctcagggttctcatt
 aaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatgggggatggaagcac
 30 agactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctaaaaatgaacagtctgcaa

actgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatggtatggactactgggggtcaaggaacctc
agtcaccgtctcctca(gatctg)

2e12 VH (amino acid sequence) (SEQ ID NO: __)

5 QVQLKESGPGGLVAPSQSLSTCTVSGFSLTGYGVNWVRQPPGKGLEWLGMWGDG
STDYNSALKSRLSITKDNSKSQVFLKMNSLQTDDTARYYCARDGYSNFHYVMD
YWGQGTSVTVSS

2e12scFv(+Restriction sites) (nucleotide sequence) (SEQ ID NO: __)

10 aagcttatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc
aatctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaagtggtgaatattatgtcacaagtt
taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctgggggtccctgcc
aggtttagtggcagtggggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttgacgttcggtggaggcaccaagctggaaatcaaacgggggtggcgggtggctcgggcggaggtggg
15 tcgggtgagcggcggaatcaggtgcagctgaaggagtcaggacctggcctgggtggcgccctcacagagcctgtccalcacatgc
accgtctcaggggtctcattaaaccggctatggtgtaaactgggtcgcagcctccaggaaagggcttgagtggtgggaatgat
atgggggtgatggaagcacagactataattcagctctcaaaccagactgagcatcaccaaggacaactccaagagccaagtttctt
aaaaatgaacagctctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatggtatggact
actgggggtcaaggaacctcagtcaccgtctcctct(gatcag)

20

2e12scFv (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPLKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYP
CQQSRKVPWTFGGGTKLEIKRGGGSGGGGSGGGGSGVQLKESGPGGLVAPSQSL
25 ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQGTSVTVSS

10A8 is anti-CD152 (CTLA-4)

10A8 VL (with L6 VK leader peptide) (nucleotide sequence) (SEQ ID NO: __)

atggattttcaagtgagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacacagtct
ccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggttggtac
caacacaagcctggaaaagggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtggaggt
gggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaacttattattgtcaacagtatgataatcttcc
5 attgacgttcggctcggggacaaagttggaataaaacgg

10A8 VL (amino acid sequence) (SEQ ID NO:___)

MDFQVQIFSLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDISLRNLEPEDIATYYCQQY
10 DNLPLTFGSGTKLEIKR

10A8 VH (no leader peptide) (nucleotide sequence) (SEQ ID NO:___)

gatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtcctctgtctctcacctgctctgtcactggctactccatcac
cagtggtttctactggaactggatccgacagtttccgggaaacaaactggaatggatgggccacataagccacgacggttaggaat
15 aactacaacctatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagtttttctgaagttgagttctgtgactactg
aggacacagctacatatttctgtgcaagacactacggttagtagcggagctatggactactgggggtcaaggaacctcagtcaccgtc
tcctctgatca

10A8 VH (amino acid sequence) (SEQ ID NO:___)

DVQLQESGPGLVKPSQSLTCSVTGYSITSGFYWNWIRQFPGNKLEWMGHISHD
GRNNYNPSLINRISITRDTSKNQFFLKLSSVTTEDTATYFCARHYGSSGAMDYWGQ
GTSVTVSS

10A8 SCFV (nucleotide sequence) (SEQ ID NO:___)

aagcttatggattttcaagtgagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
cagtcctcatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggtt
ggtaccaacacaagcctggaaaagggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagt
gaagtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaacttattattgtcaacagtatgataat
cttcattgacgttcggctcggggacaaagttggaataaaacgggggtggcgggtggctcgggcggtgggtgggtcgggtggcggc
30 ggatctgatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtcctctgtctctcacctgctctgtcactggctactc

catcaccagtgggttctactggaactggatccgacagttccgggaaacaaactggaatggatgggcccacataagccacgacggta
 ggaataactacaacccatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtga
 ctactgaggacacagctacatatttctgtgcaagacactacggtagtagcgagctatggactactggggtcaaggaacctcagtc
 accgtctcctctgatca

5

10A8 SCFV (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSFLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
 WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDISIRNLEPEDIATYYCQQY
 DNLPLTFGSGTKLEIKRGGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSV
 10 TGYSITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFL
 KLSSVTTEDTATYFCARHYGSSGAMDYWGQGTSVTVSSD

40.2.220-hmIgG1-hCD80 (nucleotide sequence) (SEQ ID NO: __)

aagcttatggattttcaagtgcagatttcagcttctgtaatacagtgcttcagtcataatgtccagaggagtcgacattgttctgactc
 15 agtctccagccaccctgtctgtgactccaggagatagagtctcttcttctgcagggccagccagagtattagcgactacttacactg
 gtatcaacaaaaatcacatgagtcctcaaggcttctcatcaaatatgcttccattccatctctgggatccctccaggttcagtgga
 gtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaatttattactgtcaacatgtgcacagcttc
 cgtggacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtgggtcgggtggcggcg
 gatctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtat
 20 gccttcacaactactggaatgcagtggtgcaagagatgccaggaaagggttgaaagtgattggctggataaacaccccactctg
 gagtgcacaaatagtagaagacttcaaggacggttgcttctcttggaaacctctgccaacactgcatatttacagataagcaacc
 tcaaagatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatgacctggcctactttgcttactggggccaag
 ggacactggctactgtctctgatctggagcccaaatctctgacaaaactcacacatccccaccgtccccagcacctgaactcctgg
 ggggatcgtcagttcttcttcccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtggtggtg
 25 gacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcggtgaggtgcataatgccaagacaaagccgcg
 ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa
 gtgcaaggtctccaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacagg
 tgtacaccctgccccatcccggtatgagctgaccaagaaccagtcagcctgacctgcctggtcaaaggcttctatccagcga
 catcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcct
 30 tcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctg
 cacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctccatcctgggccattacctta

atctcagtaaattggaattttgtgatatgctgcctgacctactgctttgcccccaagatgcagagagagaaggaggaatgagagattga
gaagggaagtgtacgccctgtataaatcgat

40.2.220-hmIgG1-hCD80 (amino acid sequence) (SEQ ID NO: __)

5 MDFQVQIFSFLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLINSVEPEDVGIYYCQHGHSF
PWTFGGGTKLEIKRGGGGSGGGGSGGGGSGIQLVQSGPELKKPGETVRISCKASG
YAFTTTGMQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAY
LQISNLKDEDTATYFCVRSNGNYDLAYFAYWGQGTLLTVSDLEPKSSDKTHTSP
10 PSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKA
DPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

15

2e12scFv- hmIgG1-CD80 fusion protein (nucleotide sequence) (SEQ ID NO: __)

aagcttatggatttcaagtcagatttcagcttctgctaatacagtcgttcagtcataatgtccagaggagtcgacattgtctcacc
aatctccagcttctttggtgtgtctctaggtcagagagccaccatctctcagagccagtgaaagtgttgatattatgtcacaagtt
taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
20 aggttttagtggcagtggtgtgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcggtggtcgggaggaggtggg
tcgggtggcggtggtatcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
accgtctcagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atggggtgatggaagcacagactataattcagcttcaaatccagactgagcatcaccaaggacaactccaagagccaagtttctt
25 aaaaatgaacagctctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggact
actggggtcaaggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtcccca
gcacctgaactcctggggggatcgtcagttctcttcccccaaaacccaaggacacctcatgatctccggacccttgaggt
cacatgcgtggtgggtgacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgc
caagacaaagccgaggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctga
30 atggcaaggagtacaagtgaaggtctccaacaaagccctccagcccccatcgagaaaaccatctccaaagccaaagggcag
ccccgagaaccacaggtgtacacctgcccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaa

ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgct
 ggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtttctcatgctcc
 gtgatgatgaggtctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaacctgctccc
 atcctgggccattacctaatactcagtaaatggaattttgtgatatgctgcctgacctactgctttgccccaaagatgcagagagagaa
 5 ggaggaatgagagattgagaagggaagtgtacgcctgtataaatcgat

2e12scFv- hmtIgG1-CD80 fusion protein (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSFLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
 10 CQQRKVPWTFGGGTKLEIKRGGGSGGGSGGGGSQVQLKESGPGLVAPSQSL
 ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMWGDGSTDYNSALKSRLSITKDNS
 KSQVFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQTSVTVSSDLEPKS
 SDKTHTSPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 15 APIEK.TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL
 SLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

10A8 scFv-hmtIgG1-CD80 (nucleotide sequence) (SEQ ID NO: __)

aagcttatggattttcaagtcagattttcagcttctgctaatacagtcctcagtcataatgtccagaggagtcgacatccagatgaca
 cagtcctccatcctcactgtctgcatctctgggaggcaaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggtt
 ggtaccaacacaagcctggaaaaggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtg
 gaagtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactattattgtcaacagtatgataat
 ctccattgacgttcggctcggggacaaagtggaaataaaacgggggtggcgggtggctcgggcgggtgggtgggtcggcggc
 25 ggatctgatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtcctctgtctctcacctgctctgtcactggctactc
 catcaccagtggtttctactggaactggatccgacagttccgggaaacaaactggaatggatgggccacataagccacgacggta
 ggaataactacaacccatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtga
 ctactgaggacacagctacatatttctgtgcaagacactacggtagtagcggagctatggactactggggtcaaggaacctcagtc
 accgtctcctctgatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggatc
 30 gtcagtcctccttccccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtgggtggacgtga
 gccacgaagaccctgaggtcaagttcaactgtgacgtggacggcgtggaggtgcataatccaagacaaagccgcgggagga

gcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaa
 ggtctccaacaaagccctcccagccccatcgagaaaaccatctcaaagccaaagggcagccccgagaaccacaggtgtaca
 ccctgcccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgctgggtcaaaggcttctatcccagcgacatcgc
 cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttct
 5 ctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
 cactacacgcagaagagcctctccctgtctccgggtaaagcggtaccttgaacctgctcccatcctgggccattaccttaattca
 gtaaattggaattttgtgatatgctgcctgacctactgctttgccccagatgcagagagagaaggaggaatgagagattgagaagg
 gaaagtgtacgcctgtataaatcgat

10 **10A8 scFv-hmIgG1-CD80 (amino acid sequence) (SEQ ID NO: __)**

MDFQVQIFSFLISASVIMSRGVDIQMTQSPSSLSASLGKVTITCKASQDIKKYIG
 WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDISIRNLEPEDIATYYCQQY
 DNLPLTFGSGTKLEIKRGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSTCSV
 TGYSITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFL
 15 KLSSVTTEDTATYFCARHYGSSGAMDYWGQGTSTVSSDLEPKSSDKTHTSPSP
 APELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
 GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
 VLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKADPS
 20 NLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

500A2-hmIgG1-CD80 (nucleotide sequence) (SEQ ID NO: __)

atgttgatacatctcagctccttgggcttttactcttctggatttcagctccagaagtgcacatagtctgactcagactccagccactc
 tgtctctaattcctggagaaagagtcacaatgacctgtaagaccagtcagaatattggcacaatcttacactggatcaccaaaaacc
 25 aaaggaggctccaagggtctcatcaagtatgcttcgcagtcattcctgggatccccctccagattcagtggcagtggttcggaaac
 agattcactctcagcatcaataacctggagcctgatgatatcggaatttactgtcaacaaagtagaagctggcctgtcacgttcg
 gtctggcaccaagctggagataaaacggggtggcgggtggctcgggcggagggtgggtcgggtggcggcgatctcaggtcaa
 gctgcagcagtcgggttctgaactagggaaacctggggcctcagtgaaactgtcctgcaagacttcaggctacatattcacagatc
 actatatttctgggtgaaacagaagcctggagaaagcctgcagtgataggaaatgtttatggtggaaatggtgtacaagctaca
 30 atcaaaaattccagggcaaggccacactgactgtagataaaatctctagcacagcctacatggaactcagcagcctgacatctgag
 gattctgccatctattactgtgcaagaaggccggtagcgacgggccatgctatggactactggggtcaggggatccaagttaccgt

ctcctctgatctggagcccaaatctctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggatcgtcagt
 cttctcttcccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacg
 aagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtac
 aacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggcttcca
 5 acaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccc
 ccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccagcgacatcgccgtggagt
 gggagagcaatgggcagccggagaaactacaagaccacgcctcccgctggtgactccgacggctccttctcctctacagca
 agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac
 gcagaagagcctctccctgtctccgggtaaaagcggatccttcgaacctgctccatcctgggccattacctaatactcagtaaagtga
 10 atttttgtgatatgctgcctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagtga
 cgcctgtataaatcgat

500A2-hmtIgG1-CD80 (amino acid sequence) (SEQ ID NO: __)

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYH
 15 QKPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPV
 TFGPGTKLEIKRGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIF
 TDHYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMEL
 SSLTSEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSDLEPKSSDKTHTSPSPAP
 ELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
 20 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
 DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKADPSNL
 LPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

25 2H7 scFv MTH(SSS)WTCH2CH3 (nucleotide sequence) (SEQ ID NO: __)

aagcttgcgcgatggattttcaagtgcagatttcagcttctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
 cccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcact
 ggtaccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagt
 gcagtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggattt
 30 taaccacccacgcttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctggtgggtgagtcgtgggcctcagtgaaagatgtctgcaaggcttctggc

tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactttactggtacttcgatgtctggggcac
 agggaccacgggtaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcccagcacctgaac
 5 tcttggggggaccgtcagcttcttctcccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtg
 gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
 ccgcgaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
 gtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaac
 cacaggtgtacaccctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatcc
 10 cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccggtgctggactccgac
 ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgatga
 ggctctgcacaaccactacacgcagaagagcctctccctgtctccggtaaataatgatctaga

2H7 scFv MTH(SSS)WTCH2CH3 (amino acid sequence) (SEQ ID NO: __)

15 MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
 20 THTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
 PGK

25

**2H7 scFv IgAH IGG WT CH2CH3 (2H7 scFv with IgA hinge and WT CH2 and CH3)
 (nucleotide sequence) (SEQ ID NO: __)**

aagcttcccgccatggatttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctct
 ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcact
 30 ggtaccagcagaagccaggatcctccccaacccctggatttatgccccatccaacctggcttctggagtccctgctcgttctcagtg
 gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggattt

taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
 tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 5 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcac
 agggaccacggtcaccgtctctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctca
 tgcgcacctgaactcctggggggaccgtcagttctctctccccccaaaacccaaggacacccctcatgatctcccgaccctga
 ggtcacatgcgtgggtgggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataa
 tgccaagacaaagccgcggggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggc
 10 tgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggc
 agccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
 aaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccggtg
 ctggactccgacggctccttctctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
 cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

15

2H7 scFv IgAH IGG WT CH2CH3 (amino acid sequence) (SEQ ID NO:___)

MDFQVQIFSFLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 20 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSDQVPSTPPTP
 SPSTPPTPSPSCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 25 PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL
 SLSPGK

2H7 scFv IgAH IgACH2CH3 (2H7 scFv IgAhinge and IgA CH2 and CH3) (nucleotide sequence) (SEQ ID NO:___)

30 aagcttgcgccatggattttcaagtcagattttcagcttctgctaatacagtcgctcagtcataattgccagaggacaaattgttctct
 cccagctctccagcaatcctgtctgcacatccaggggagaagggtcacaatgacttgaggccagctcaagtgttaagtacatgcact

ggtaccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
 gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagctggtagggcctggggcctcagtgaagatgtcctgcaaggcttctggc
 5 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggctatttctgtgcaagagtgggtgactatagtaactcttactggacttctgatgtctggggcac
 agggaccacggtcaccgtctcttctgatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctccc
 tcatgtctgccacccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacact
 10 gaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgacctg
 gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgcactgc
 tgcctaccccgagtccaagaccccgtaaccgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccg
 ccgccgtcggaggagctggccctgaacgagctgggtgacgtgacgtgcctggcacgtggcttcagccccaaggatgtgctggtt
 cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcacatccggcaggagcccagccaggggcacca
 15 ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggggccacg
 aggcctgccgctggccttcacacagaagaccatcgaccgcttggcggttaaccacccatgtcaatgtgtctgtgtcatggcg
 gaggtggacggcacctgctactgataatctaga

2H7 scFv IgAH IgACH2CH3 (2H7 scFv IgA hinge and IgA CH2 and CH3) (amino
acid sequence) (SEQ ID NO: __)
 MDFQVQIFSLLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 25 TAYMQLSSLTSEDSAVYFCARVYYSNSYWFYFDVWGTGTTVTVSSDQVPVSTPPT
 PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWTPSSG
 KSAVQGPPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
 GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLT
 WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
 30 GKPTHVNVSVVMAEVDGTCY

IgA hinge-CH2-CH3 (Human IgA tail, full length) (nucleotide sequence) (SEQ ID NO:___)

5 tgatcagccaggtccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
 ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
 10 gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
 tccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgcaactgctgcctaccccgagtccaagaccc
 cgctaacccgccaccctctcaaaatccggaaacacattccggcccagaggtccacctgctgccggccgctcgaggagctggccc
 tgaacgagctggtgacgtgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg
 agctgccccgcgagaagtacctgacttgggcatcccgccaggagccagccagggcaccaccaccttcgctgtgaccagcata
 15 ctgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcac
 acagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgtgtcatggcggaggtggacggcacctgctact
 gataatctaga

IgA hinge-CH2-CH3 (Human IgA tail, full length) (amino acid sequence) (SEQ ID NO:___)

15 DQVPVSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
 TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
 PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQ
 20 ELPREKYLTWASRQEPSQGTTTFAVTSILRVAEDWKKGDTFSCMVGHEALPLAF
 TQKTIDRLAGKPTHVNVSVVMAEVDGTCY

Human J Chain (nucleotide sequence) (SEQ ID NO:___)

25 agatctcaagaagatgaaaggattgttcttggtgacaacaaatgtaagtgtgcccggattacttcaggatcatccgttctccgaaga
 tcctaatagggacattgtggagagaaacatccgaattattgttctctgaacaacaggagagaatatctctgatccacctcaccattga
 gaaccagatttgtgtaccatttgtctgacctcagctgtaaaaaatgtgatcctacagaagtggagctggataatcagatagtactgct
 acccagagcaatatctgtgatgaagacagtgtacagagacctgtacacttatgacagaaacaagtgtacacagctgtgtgtccc
 actcgtatatggtggtgagacaaaaatggtggaaacagccttaacccagatgcctgctatcctgactaatctaga

Human J Chain (amino acid sequence) (SEQ ID NO:___)

RSQEDERIVLVDNKCKCARITSRIIRSSDPNEDIVERNIRIIVPLNNRENISDPTSPLR
TRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAV
VPLVYGGETKMOVETALTPDACYP

- 5 **4 carboxy terminal amino acids deleted from IgA CH3 (amino acid sequence) (SEQ ID NO: __)**
GTCY

- 10 **IgAH IgAT4** (human IgA tail, truncated (3T1)-(missing last 4 amino acids from carboxy terminus) (nucleotide sequence) (SEQ ID NO: __)
tgatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgctgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaacatgggaagaccttcacttgactgctgcctaccccgagtccaagacc
15 cgctaaccgccaccctctcaaaatccggaacacattccggcccgaggtccacctgctgccgccgccgctggaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagcccaaggatgtgctggctgctggctgcaggggtcacagg
agctgccccgcgagaagtacgtgactgggcatcccgccaggagcccagggcaccaccaccttcgctgtgaccagcata
ctgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatgggtgggccacgaggccctgccgctggcctcac
acagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgtgtcatggcgagggtggactgataatctaga

- 20 **IgAH IgAT4 (amino acid sequence) (SEQ ID NO: __)**
DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQ
25 ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGKPHTVNVSVVMAEVD

2H7 scFv IgAH IgAT4 (2H7 scFv IgA 3T1 construct; truncates the CH3 domain at the 3'end) (nucleotide sequence) (SEQ ID NO: __)

aagcttgccgcatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctct
 cccagcttccagcaatcctgtctgcatctccaggggagaagggtcacaatgacttgaggggccagctcaagtgttaagttacatgcact
 ggtaccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtg
 gcagtggtgtgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggttt
 5 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggagggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagctggtaggcctggggcctcagtgaaagatgtcctgcaaggcttctggc
 tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaat
 ggtgatacttctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcac
 10 agggaccacggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccc
 tcatgctgccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacact
 gaccggcctgagagatgcctcaggtgtcaccttcacctggagcgcctcaagtgggaagagcgctgttcaaggaccacctgaccgt
 gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgc
 tgcctaccccgagtccaagaccccgtaaccgccaccctctcaaaatccggaaacacattccggcccagggtccacctgctgccg
 15 ccgccgtcggaggagctggccctgaacgagctggtagcgtgacgtgcctggcacgtggcttcagccccaaagatgtgctggtt
 cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcaccccgagggagccagccagggcacca
 ccaccttcgctgtgaccagcactatgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggggcacg
 aggcctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcattggcg
 gaggtggactgataatctaga

20

2H7 scFv IgAH-T4 (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILASPGKEVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPS NLAGV PARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 25 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQVPVSTPPT
 PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
 KSAVQGPDRDLGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
 GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLT
 30 WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
 GKPTHVNVSVVMAEVD

14 amino acids deleted from IgAH-T4 (so that total of 18 amino acids deleted from wild type IgA CH3) (amino acid sequence) (SEQ ID NO:___)

PTHVNVSVVMAEVD

5

IgAH IgA-T18 (human IgA Tail truncated, 3T2) (nucleotide sequence) (SEQ ID NO:___)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
ctgcaccgaccggccctcaggacgtgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
10 gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaacatgggaagaccttcactgctgctaccccgagtccaagacc
cgctaaccgccaccctctcaaaatccggaacacattccggcccgaggtccacctgtgccgccgccgtcggaggagctggccc
tgaacgagctggtgacgtgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggctgcaggggtcacagg
agctgccccgcgagaagtacctgacttgggcacccggcaggagcccagccagggcaccaccaccttcgtgtgaccagcata
15 ctgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgtggccttcac
acagaagaccatcgaccgcttggcgggtaaa

IgAH IgA-T18 (amino acid sequence) (SEQ ID NO:___)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
20 TFTWTPSSGKSAVQGPPDRDLGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVA AEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGK

25 **2H7 scFv IgAH IgAT18** (human IgA Tail truncated, 3T2) (nucleotide sequence) (SEQ ID NO:___)

aagcttgccgccatggatttcaagtgcagatttccagcttctgctaatacgtgcttcagtcataattgccagaggacaaattgttctc
cccagctctccagcaatcctgtctgcatctccaggggagaaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcact
ggtagcagcagaagccaggatcctccccaaacctggattatgccccatccaacctggcttctggagtcctgctcgttcagt
30 gcagtgggtctgggaccttactctctcacaatcagcagagtgaggagctgaagatgctgccacttattactgccagcagtgaggatt
taaccacccacgttcggtgctgggaccaagctggagctgaagatggcggtggtcggcggtggtggatctggaggaggtg

ggagctctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagtcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggctctatttctgtgcaagagtgggtgtactatagtaactcttactggtacttcgatgtctggggcac
 5 agggaccacggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccc
 tcatgtgccacccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacact
 gaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgt
 gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacatgggaagaccttcacttgcactgc
 tgctaccccgagtccaagaccccgtaaccgccaccctctcaaatccggaaacacattccggccccgaggtccacctgctgccg
 10 ccgccgtcggaggagctggccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagccccaaggatgtgctggtt
 cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcacca
 ccaccttcgtgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggggccacg
 aggccttgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaa

15 **2H7 scFv IgAH IgAT18 (amino acid sequence) (SEQ ID NO: __)**
 MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 20 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQVPVSTPPT
 PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWPSSG
 KSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
 GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLT
 WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
 25 GK

CTLA-4 IgG WTH WTCH2CH3 (human-oncoMLP-CTLA4EC-hIgGWT)
(nucleotide sequence) (SEQ ID NO: __)

gcaacctacatgatggggaatgagttgaccttctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcact
 30 atccaaggactgagggccatggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcatag
 gcaacggaacccagatttatgtaattgatccagaaccgtgccagattctgatcaacccaaatctgtgacaaaactcacacatgcc

caccgtgccagcacctgaactcctggggggaccgtcagcttctcttcccccaaaaccaaggacaccctcatgatctccgg
 acccctgaggtcacatgcgtgggtgggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggag
 gtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
 gactggctgaatggcaaggagtacaagtcaagggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagcc
 5 aaagggcagccccgagaaccacaggtgtacaccctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctg
 cctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgc
 ctcccgctgtggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctt
 ctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

10 **CTLA-4 IgG WTH WTCH2CH3 (amino acid sequence) (SEQ ID NO:___)**

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
 TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
 MDTGLYICKVELMYPPPYLIGINGTQIYVIDPEPCPDSQPKSCDKTHTCPPCPAP
 ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
 15 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
 DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

20 **Human OncoM leader Peptide+CTLA4 EC (BclI) (nucleotide sequence) (SEQ ID NO:___)**

atgggggtactgctcacacagaggacgctgctcagctgtgctcctgcactcctgtttccaagcatggcgagcatggcaatgcacgt
 ggcccagcctgctgtgtgactggccagcagccgaggcatgccagcttctgtgtgagtatgcatctccaggcaaagccactgag
 gtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
 tctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctactatccaaggactgagggccatggacacggg
 25 actctacatctgcaagggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatcc
 agaaccgtgccagattctgatcaa

30 **Human OncoM leader Peptide+CTLA4 EC (amino acid sequence) (SEQ ID NO:___)**

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
 TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
 MDTGLYICKVELMYPPPYLIGINGTQIYVIDPEPCPDSQ

Human OncoM leader (nucleotide sequence) (SEQ ID NO:___)

atgggggtactgctcacacagaggacgctgctcagtcctgctcctgtttccaagcatggcgagcatg

5 Human OncoM leader (amino acid sequence) (SEQ ID NO:___)

MGVLLTQRTL LSLVLALLFPSM

Human CTLA4 EC (no LP) (nucleotide sequence) (SEQ ID NO:___)

gcaatgcacgtggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggca
 10 aagccactgagggtccgggtgacagtgttcggcagggtgacagccagggtgactgaagtctgtcggcaacctacatgacgggga
 atgagttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgaggggcca
 tggacacgggactctacatctgcaagggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttat
 gtaattgatccagaaccgtgccagattct

15 Human CTLA4 EC (no LP) (amino acid sequence) (SEQ ID NO:___)

AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYM
 TGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLIGING
 TQIYVIDPEPCPDS

20 Human CTLA4 IgG MTH (SSS) MTCH2CH3 (nucleotide sequence) (SEQ ID NO:___)

atgggggtactgctcacacagaggacgctgctcagtcctgctcctgtttccaagcatggcgagcatggcaatgcacgt
 ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
 gtccgggtgacagtgttcggcagggtgacagccagggtgactgaagtctgtcggcaacctacatgatggggaatgagttgacct
 25 tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgaggggccatggacacggg
 actctacatctgcaagggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatcc
 agaaccgtgccagattctgatcaacccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggg
 gatcgtcagtccttcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggac
 gtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcggga
 30 ggagcagtacaacagcacgtaccgtgtgtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagt

caaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgt
 acaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacat
 cgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctt
 cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcac
 5 aaccactacacgcagaagagcctctccctgtctccgggtaaatga

Human CTLA4 IgG MTH (SSS) MTCH2CH3 (amino acid sequence) (SEQ ID NO: __)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
 10 TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
 MDTGLYICKVELMYPPPYLIGINGTQIYVIDPEPCPDSQPKSSDKTHTSPPSPAP
 ELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
 15 DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

CTLA-4 IgAH IgACH2CH3 (human-oncoMLP-CTLA4EC-IgA) (nucleotide sequence) (SEQ ID NO: __)

atgggggtactgctcacacagaggacgctgctcagctggtccttgactcctgtttccaagcatggcgagcatggcaatgcacgt
 20 ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
 gtccgggtgacagtgttcggcaggctgacagccagggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
 tcctagatgattccatctgcacgggcacctccagtggaatcaagtgaacctcactatccaaggactgagggccatggacacggg
 actctacatctgcaaggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatcc
 agaaccgtgccagattctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgct
 25 gccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccgg
 cctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgacctgacctct
 gtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgctgcctac
 cccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccg
 tcggaggagctggccctgaacgagctggtgacgctgacgtgctggcacgtggcttcagccccaaggatgtgctggttcgctgg
 30 ctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccgccagggagccagccagggcaccaccacctt
 cgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccc

tgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgttgcacggcgaggtg
gacggcacctgctactgataatctaga

CTLA-4 IgAH IgACH2CH3 (amino acid sequence) (SEQ ID NO: __)

5 MGVLLTQRTLSSLVLALLFSPMASMAMHVAQPAVVLAASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYLIGIGNGTQIYVIDPEPCPDSQVPVSTPPTSPSTPPTP
SPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQGPP
DRDLCGCYSVSSVLPGCAEPWNHGTFTCTAAYPESKTPLTATLSKSGNTFRPEVH
10 LLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQ
GTTTFAVTSILRVAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPHTVNVS
VVMAEVDGTCY

CTLA-4 IgAH IgA-T4 (human-oncoMLP-CTLA4EC-IgA3T1) (nucleotide sequence)
(SEQ ID NO: __)

15 atgggggtactgctcacacagaggacgctgctcagctctggctcctgcactcctgttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgctgtgtgactggccagcagccgaggcatgccagcttctgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgtctggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagtgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
20 actctacatctgcaaggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatcc
agaaccgtgccagattctgatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgct
gccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccgg
cctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctct
gtggctgtacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgtgcctac
25 cccgagtccaagaccccgctaaccgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccgccgccg
tcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctgg
ctgcaggggtcacaggagctgccccgcgagaagtacgtgactgggcatcccgccaggagcccagggcaccaccacctt
cgctgtgaccagcactgctgcgctggcagccgaggactggaagaagggggacacctctcctgcatggtggccacgaggccc
tgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgttgcacggcgaggtg
30 gactgataatctaga

CTLA-4 IgAH IgA-T4 (amino acid sequence) (SEQ ID NO: __)

MGVLLTQRTL LSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
 TEVRVTVL RQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
 MDTGLYICKVELMYPPPYL GIGNGTQIYVIDPEPCPDS DQPVPSTPPTPSPSTPPTP
 5 SPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTF TWTWPSSGKSAVQGPP
 DRDLCGCYSVSSVLP GCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVH
 LLPPPSEELALNELVTLTCLARGFSPKDV LVRWLQGSQELPREKYLTWASRQEPSQ
 GTTTF AVTSILRVAAEDWKKGDTFSCMV GHEALPLAFTQKTIDRLAGKPTHVNV
 VVMAEVD

10

human IgG1 CH2 with 238 mutation pro→ser (nucleotide sequence) (SEQ ID NO: __)

cctgaactcctggggggatcgtcagtcctcctcctcccccaaaaccaaggacaccctcatgatctccggacccctgaggtcac
 atgcgtgggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgcaa
 15 gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
 gcaaggagtacaagtgcaaggctccaacaaagccctccagcccccatcgagaaaaccatctccaaagccaaag

15

human IgG1 CH2 with 238 mutation pro→ser (amino acid sequence) (SEQ ID NO: __)

20 PELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

Amino acids surrounding Pro to Ser in CH2 (amino acid sequence) (SEQ ID NO: __)

PAPELLGGPS

25

Amino acids surrounding Pro to Ser in CH2 (amino acid sequence) (SEQ ID NO: __)

PAPELLGGSS

hIgE3BB (leaves an open reading frame at end of gene to read into transmembrane and cytoplasmic tail domain attached at either the BamHI or SfuI sites) (nucleotide sequence) (SEQ ID NO: __)

gtt gtt ttc gaa gga tcc gct tta ccg gga ttt aca gac acc gct cgc tgg

5

human IgE Fc (CH2-CH3-CH4) ORF (nucleotide sequence) (SEQ ID NO: __)

tgatcacgtctgctccagggaacttcaccccgccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttcccccg
accatccagctcctgtgcctcgtctctgggtacacccagggaactatcaacatcacctggctggaggacgggcagggtcatggacg
tggactgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacctcagccagaagcactggc
10 tgtcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaagtgtgcagattccaacc
gagaggggtgagcgcctacctaagccggcccagcccgttcgacctgtcatccgaagtcgccacgatcacctgtctggtggtg
gacctggcaccgcgaagggggaccgtgaacctgacctgggtcccgggccagtgggaagcctgtgaaccactccaccagaaagg
aggagaagcagcgcgaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgagggggagaccta
ccagtgcagggtgacccacccccacctgccagggccctcatcggtccacgaccaagaccagcggcccgctgtctgccccg
15 gaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaactcatgcct
gaggacatctcgggtgcagtgggtgcacaacgaggtgcagctcccgacgccgggcacagcacgacgcagccccgaagacc
aagggtccggcttctcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
tccatgaggcagcgagccctcacagaccgtccagcgagcgggtgtctgtaaataccggtaaagcggatccttcgaa

20 **human IgE Fc (CH2-CH3-CH4) ORF** (amino acid sequence) (SEQ ID NO: __)

DHVCSDFTPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
PRGVSAYLSRPSFDLFIKSPITITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE
EKQRNGTLTVTSTLPVGTDRDWEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
25 VYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
GSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

IFhIgGwtBcl5 (nucleotide sequence) (SEQ ID NO: __)

gtt gtt tga tca gga gcc caa atc ttg tga caa aac tca cac atg ccc acc gtg ccc agc acc (63 mer)

30

HuIgGMHWC (sense, 5' primer for mutating wild type hinge CCC to mutant SSS)
(nucleotide sequence) (SEQ ID NO:___)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa ctc ctg
ggg gga ccg tca gtc ttc c

5

1D8 VH (nucleotide sequence) (SEQ ID NO:___)

cagggtgcagctgaaggaggcaggacctggcctgggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcatt
aaccagcgaatgggtgtacactggattcgacagcctccaggaaagggctggaatggatgggaataatatattatgatggaggcaca
gattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagtttcttaaaaatcaacagctgcgaaa
ctgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcaggtcacagctcctct

10

1D8 VH (no leader) (amino acid sequence) (SEQ ID NO:___)

QVQLKEAGPGLVQPTQTLSLTCTVSGFSLTSDGVHWIRQPPGKGLEWMGIYYDG
GTDYNSAIKSRLSISRDTSKSQVFLKINSLQTDDTAMYVCARIHFDYWGQGVMT
VSS

15

1D8 VL (no leader) (nucleotide sequence) (SEQ ID NO:___)

gacattgtgctcactcagctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtg.
aagttacatgtactgggtaccagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagtcca
aatcgcttcagtggcagtggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtca
gcagtgaggtagtactccgctcacgttcgggtctgggaccaagctggagatcaaacgg

20

1D8 VL (amino acid sequence) (SEQ ID NO:___)

DIVLTQSPTTIAASPGKVTITCRASSSVSYMYWYQQKSGASPKLWIYDTSKLSG
VPNRFSGSGSGTSYSLAINTMETEDAATYYCQQWSSTPLTFGSGTKLEIKR

25

1D8 scFv (nucleotide sequence) (SEQ ID NO:___)

aagcttatggattttcaagtgcagatttcagcttcctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggg
accagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagtccaaatcgcttcagtgga

30

gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgaggtagtact
 ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtcgggtggcggcg
 gatctcaggtgcagctgaaggaggcaggacctggcctgggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
 ctcaataaccagcgatgggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
 5 cacagattataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagttttctaaaaatcaacagtctg
 caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcattgggtcacagtctcctctgatc
 a

1D8 scFv (amino acid sequence) (SEQ ID NO: __)

10 MDFQVQIFSFLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 INSLQTDDTAMYYCARIHFDYWGGQGMVTVSS

15

1D8 scFv IgG WTH WTCH2CH3 (nucleotide sequence) (SEQ ID NO: __)

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactc
 agtctccaacaaccatagctgcatctccaggggagaagggtcacatcacctgccgtgccagctccagtgtaatgtacatgtactgggt
 accagcagaagtcaggcgctcccctaaactctggatttatgacacatccaagctggcttctggagtccaaatcgcttcagtggca
 20 gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgaggtagtact
 ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtcgggtggcggcg
 gatctcaggtgcagctgaaggaggcaggacctggcctgggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
 ctcaataaccagcgatgggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
 cacagattataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagttttctaaaaatcaacagtctg
 25 caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcattgggtcacagtctcctctgatc
 aggagcccaaactctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagttctctcttc
 ccccaaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtgggtgacgtgagccacgaagacct
 gaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccggggaggagcagtacaacagcac
 gtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagcc
 30 ctccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatccc
 ggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaggcttctatccagcgacatcgccgtggagtgggagag

caatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaag
agcctctccctgtctccgggtaaatgatctaga

5 **1D8 scFv IgG WTH WTCH2CH3** (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSFLISASVMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWYDTSKSLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
10 INSLQTDDTAMYYCARIHFDYWGQGMVTVSSDQEPKSCDKTHTCPPCPAPPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

15

1D8 scFv IgG MTH MTCH2CH3-CD80 (nucleotide sequence) (SEQ ID NO: __)

aagcttatggatttcaagtcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactc
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accagcagaagtcaggcgcctcccctaaactctgattatgacacatccaagctggcttctggagttccaaatcgcttcagtgga
20 gtgggtctgggacctcttattctctgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgaggtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaagggtggcggtggctcggcggtgggtgggtcgggtggcggcg
gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
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cacagattataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaatcaacagtctg
25 caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcaggtcacagtctcctctgatct
ggagcccaaattcttgacaaaactcacacaagcccaccgagcccagcacctgaactcctggggggatcgtcagttcttctctcc
ccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagaccctg
aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgaggaggagcagtaacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaagggtctccaacaaagccc
30 tcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccgg
gatgagctgaccaagaaccagtcagcctgacctgctggtcaaaaggcttctatcccagcgacatcgccgtggagtgaggagagc

aatgggcagccggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttctctctacagcaagctcaccg
 tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
 gcctctccctgtctccgggtaaagcggatccttgaacctgctccatcctgggccattacctaatactcagtaaatggaattttgtga
 tatgctgcctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgt
 5 ataaatcgata

1D8 scFv IgG MTH MTCH2CH3-CD80 (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSFLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 QQKSGASPKLWIYDTSKLASGVNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 10 SSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 INSLQTDDTAMYYCARIHFDYWGGQVMVTVSSDLEPKSSDKTHTSPSPAPELLG
 GSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 15 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKADPSNII LPSW
 AITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

1D8 scFv IgG WTH WTCH2CH3-CD80 (nucleotide sequence) (SEQ ID NO: __)

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
 agtctccaacaaccatagctgcatctccaggggagaagggtcaccatcacctgccgtgccagctccagtgttaagtacatgtactggt
 accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagtccaaatcgcttcagtgga
 gtgggtctgggaccttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgaggtagtact
 ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtggtgggtcgggtggcggcg
 25 gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
 ctattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
 cacagattataattcagcaattaaatccagactgagcatcagcaggggacacctccaagagccaagtttcttaaaatcaacagtctg
 caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcattgtcacagtctcctctgatct
 ggagcccaaactctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagcttctccttcc
 30 ccccaaaaccaaggacacctcatgatctccggaccttgagggtcacatgcgtgggtgggtggacgtgagccacgaagacctg
 aggtcaagttcaactggtagtgacggcggtggaggtgcataatgccaaagaccgcggggaggagcagtacaacagcacg

taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctccaacaagccc
 tcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatccggg
 gatgagctgaccaagaaccaggtcagcctgacctgcttggtaaaggcttctatcccagcgacatcgccgtggagtgggagagc
 aatgggcagccggagaaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctcaccg
 5 tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
 gcctctccctgtctccgggtaaagcggatccttgaacctgtcccatcctgggccattaccttaatctcagtaaattgtga
 tatgctgcctgacctactgcttggcccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgt
 ataatcgata

- 10 **1D8 scFv IgG WTH WTCH2CH3-CD80 (amino acid sequence) (SEQ ID NO: __)**
 MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 15 INSLQTDDTAMYYCARIHFDYWGGQVMVTVSSDLEPKSCDKTHTCPPCPAPELLG
 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKADPSNLLPSW
 20 AITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

Anti-human CD3 scFv WTH WTCH2CH3 (nucleotide sequence) (SEQ ID NO: __)

- aagcttatggatttcaagtgcagatttcagcttctgctaatacgtgcttcagtcataatgtccagaggagtcgacatccagatgaca
 cagactacatcctcctgtctgcctctctgggagacagagtcaccatcagttgcagggaagtcaggacattcgcaattattaaact
 25 ggtatcagcagaaaccagatggaactgttaactcctgatctactacacatcaagattacactcaggagtcctcatcaaggttcagt
 gcagtgggtctggaacagattattctctcaccattgccaacctgcaaccagaagatattgccactactttgccaacagggtataac
 gcttccgtggacgttcggtggaggcaccaaactggaacaaacgggagctcggtggcggtggctcgggcgggtgggtgggtcgg
 gtggcgggcgatctatgatgaggtccagctgcaacagctctggacctgaactggtgaagcctggagcttaatgtcctgcaaggc
 ctctggttactcattcactggctacatcgtaactggctgaagcagagccatggaaagaaccttgagtggattggacttattaatccat
 30 acaaaggtcttactacataaccagaaattcaagggaaggccacattaactgtagacaagtcacagcacagcctacatggag
 ctctcagctgacatctgaagactctgcagctctattactgtgcaagatctgggtactatggtgactcggactggacttgatgtctgg

ggcgcaggaccacggtcaccgtctctctgatcaggagcccaaatcttgacaaaactcacacatgccaccgtgccagcac
 ctgaactcctggggggaccgtcagtcctctctcccccaaaacccaaggacaccctcatgatctccggaccctgaggtcaca
 tgcgtgggtggacgtgagccacgaagaccctgaggtcaagttcaactggtagtgacggcggtggaggtgcataatgccaag
 acaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatgg
 5 caaggagtacaagtgaaggctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagcccc
 gagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccagggtcagcctgacctgcctgggtcaaaggct
 tctatcccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtggac
 tccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
 gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

10

Anti-human CD3 scFv WTH WTCH2CH3 (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVIMSRGVDIQMTQTSSLSASLGDRVTISCRASQDIRNYLN
 WYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTIANLQPEDIATYFCQQ
 GNTLPWTFGGGTKLVTKRELGGGSGGGSGGGGSIDEVQLQQSGPELVKPGAS
 15 MSCKASGYSFTGYIVNWLKQSHGKNLEWIGLINPYKGLTTYNQKFKGKATLTVD
 KSSSTAYMELLSLTSEDSAVYYCARSGYYGDSWDYFDVWGAGTTVTVSSDQEPK
 SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
 NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
 LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
 20 GQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQ
 KSLSLSPGK

2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3 (2h7-40.2.220Ig + restriction sites)
(nucleotide sequence) (SEQ ID NO: __)

25 aagcttgccgcatggatttcaagtgcagatttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctct
 ccagctcagcaatcctgtctgcatctccaggggagaagggtcacaatgacttgaggccagctcaagtgaattacatgcact
 ggtaccagcagaagccaggatcctccccaacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtg
 gcagtggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggttt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagtgggcgtggctcgggcgggtggatctggaggaggtg
 30 ggagctctcaggcttatctacagcagctctggggctgagctggtaggcctggggcctcagtgagatgtcctgcaaggcttctggc
 tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat

ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
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 agggaccacggtcaccgtctcttctgatcaatccaactctgaagaagcaaaagaggaggccaaaaaggaggaagccaaga
 aatctaacagcgtcgacattgttctgactcagctccagccaccctgtctgtgactccaggagatagagtctctcttctcagggcc
 5 agccagagtattagcgactacttacactggtatcaacaaaaatcacatgagtctccaaggcttctcatcaaatatgcttcccattccatc
 tctgggatccccctcaggttcagtggtcagtggtcaggttcagattcactctcagatcaacagtgtggaacctgaagatgttgaa
 ttattactgtcaacatgggtcacagcttccgtggacgttcggtggaggccaaagctggaaatcaaacgggggtggcggtggctcg
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 caggatctcctgcaaggcttctgggtatgccttcacaactactggaatgcagtgggtgcaagagatgccaggaaagggttgaagt
 10 ggattggctggataaacacccccactctggagtgccaaaatagtgaagacttcaaggacggttgccttctcttggaaacctctgc
 caacactgcatatttacagataagcaacctcaaagatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatga
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 gaccttgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcggtgga
 15 ggtgcataatgccaagacaaagccgcgaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcacca
 ggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccaguccccatcgagaaaacaatctccaaagc
 caaagggcagccccgagaaccacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacct
 gcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacg
 cctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtct
 20 tctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7-antiCD40 scFv MTH (SSS) MTCH2WICH3 (2H7-40.2.220Ig) (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSFLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 25 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKGGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVYYYSNSYWFYFDVWGTGTTVTVSSDQSNSEEAK
 KEEAKKEEAKKSNSVDIVLTQSPATLSVTPGDRVSLSCRASQSIDYHWHYQQKSH
 30 ESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSPWTFGG
 GTKLEIKRGGGGSGGGGSGGGGSIQLVQSGPELKKPGETVRISCKASGYAFTTTG
 MQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAYLQISNLKD

EDTATYFCVRSGNGNYDLAYFAYWGQGLVTVSDQEPKSSDKTHTSPSPAPELL
 GGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
 FREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
 PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
 5 GSFFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

5B9 VH (includes the VH leader peptide) (nucleotide sequence) (SEQ ID NO: __)

atggctgtcttggggctgctcttctgcctggtagacattccaagctgtgcctatcccaggtagcagctgaagcagtcaggacctggcc
 tagtgcagtcctcacagagcctgtccatcacctgcacagctctgtgtttctcattaactacctatgctgtacactgggttcgccagtctc
 10 caggaaagggctctggagtggtgggagtgatggagtggtggaatcacagactataatgcagctttcatatccagactgagcatc
 accaaggacgattccaagagccaagtttctttaaataaacagctctgcaacctaataacacagccatttattactgtgccagaaatg
 ggggtgataactacccttattactatgctatggactactggggtaaggaacctcagtcaccgtctcctca

5B9 VH (minus the leader) (nucleotide sequence) (SEQ ID NO: __)

15 caggtgcagctgaagcagtcaggacctggcctagtagcagtcctcacagagcctgtccatcacctgcacagctctgtgtttctcatta
 actacctatgotgtacactgggttcgccagtcctcaggaaagggctctggagtggtgggagtgatggagtggtggaatcacaga
 ctataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagctctgcaaccta
 atgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtaaggaacctca
 gtcaccgtctcctca

20

5B9 VH (includes leader peptide) (amino acid sequence) (SEQ ID NO: __)

MAVLGLLFCLVTFPSCVLSQVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWV
 RQSPGKGLEWLGVWISGGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIY
 YCARNGGDNYPYYYAMDYWGQGSVTVSS

25

5B9 VH (no leader peptide) (amino acid sequence) (SEQ ID NO: __)

QVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWVVRQSPGKGLEWLGVWISGGI
 TDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDY
 WGQGSVTVSS

30

5B9 VL (nucleotide sequence) (SEQ ID NO: __)

atgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtgatgacgcaggctgcattctc
 caatccagtcactcttggaaacatcagcttccatctcctgcaggcttagtaagagtcctctacatagtaatggcatcacttattgtattgg
 tatctgcagaagccaggccagctcctcagctcctgattatcagatgtccaacctgcctcaggagtcaggaggttcagtagca
 5 gtgggtcaggaactgatttcacactgagaatcagcagagtgagggtgaggatgtgggtgtttattactgtgctcaaatctagaact
 tccgctcacgttcggtgctgggaccaagctggagctgaaacgg

5B9 VL (amino acid sequence) (SEQ ID NO: __)

MRFSAQLLGLLVLPWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLSHNGITY
 10 LYWYLQKPGQSPQLLIYQMSNLASGVDPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
 AQNLELPLTFGAGTKLELKR

5B9 scFv (nucleotide sequence) (SEQ ID NO: __)

aagcttgcgcgatgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtgatgacgca
 15 ggctgcattctccaatccagtcactcttggaaacatcagcttccatctcctgcaggcttagtaagagtcctctacatagtaatggcatca
 cttattgtattggtatctgcagaagccaggccagctcctcagctcctgattatcagatgtccaacctgcctcaggagtcaggagca
 gggtcagtagcagtggtcaggaactgatttcacactgagaatcagcagagtgagggtgaggatgtgggtgtttattactgtgctc
 aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacgggggtggcgggtggctcgggcgggtgggtgggt
 cgggtggcggcggtatcgtcacagggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
 20 gcacagtctctggtttctattaactacatatgctgtacactgggttcgccagctctccaggaaagggctggagtggctgggagtgat
 atggagtgggtgaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttcttt
 aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
 ctactgggtgcaaggaacctcagtcaccgtctcctct

25 5B9 scFv (amino acid sequence) (SEQ ID NO: __)

MRFSAQLLGLLVLPWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLSHNGITY
 LYWYLQKPGQSPQLLIYQMSNLASGVDPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
 AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSL
 ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
 30 SQVFFKMNSLQPNDAIYYCARNGGDNYPIYYAMDYWGQGTSTVTVSS

5B9 scFv-hmIgG1-hCD80 (nucleotide sequence) (SEQ ID NO: __)

aagcttcccgcctagaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
 ggctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
 5 cttatttgattggtatctgcagaagccaggccagtcctcctcagctcctgatttatcagatgtccaacctgcctcaggagtcccagaca
 ggttcagtagcagtggtcaggaactgattcacactgagaatcagcagagtgaggctgaggatgtgggtgtttattactgtgctc
 aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcgggcgggtgggtgggt
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 10 atggagtgggtggaatcacagactataatgcagcttccatccagactgagcatcaccaaggacgattccaagagccaagttttctt
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 cagcacctgaactcctgggggatcgtcagcttctcctctccccccaaaacccaaggacacctcatgatctcccgaccctgag
 gtcacatgcgtggtggtggacgtgagccacgaagacctgaggtcaagttcaacttggtacgtggacggcgtggaggtgcataat
 15 gccaaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactggct
 gaatggcaaggaglacaaagtgaaggtctccaacaaagccctccagcccccatcgagaaaaccatctcaaagccaaagggc
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 aaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccggtg
 ctggactccgacggctccttctcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
 20 cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaagcggatccttgaacctgctcc
 catcctggggccattacctaatactcagtaaatggaattttgtgatatgctgcctgacctactgctttgccccaaagatgcagagagaga
 aggagggaatgagagattgagaagggaagtgtacgccctgtataaatcgatactcgag

5B9 scFv-hmIgG1-hCD80 (amino acid sequence) (SEQ ID NO: __)

25 MRFSAQLLGLLVLPWGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHNSNGITY
 LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
 AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSL
 ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSSGGITDYNAAFISRLSITKDDSK
 SQVFFKMNSLQPNDAIYYCARNGGDNYPPYYAMDYWGQGTSTVTVSSDLEPKSS
 30 DKHTHTSPSPAPPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
 YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
 PIEKTISKAKGQPREPQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP

ENNYKTTTPVLDSDFSFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLS
LSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2e12 scFv WTH CH2 CH3 (2e12 scFv-WthIgG-CD80) (nucleotide sequence) (SEQ

5 ID NO:)

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10 aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtcgggcgagggtggg
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20 ccccgagaaccacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtgtaaa
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25 ggaggaatgagagattgagaagggaaggtgtacgccctgtataaatcgat

2e12 scFv WTH CH2 CH3 (2e12 scFv-WthIgG-CD80) (amino acid sequence) (SEQ
ID NO:)

MDFQVQIFSLLISASVMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
30 LMQWYQQKPGQPPLKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYP
CQQRKVPWTFGGGTKLEIKRGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMTWGDGSTDYNSALKSRLSITKDNS

KSQVFLKMNSLQTDDTARYYCARGYSNFHYVMDYWGQGSVTVSSDLEPKS
 CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 5 PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSL
 SLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2H7-human IgE Fc (CH2-CH3-CH4) (nucleotide sequence) (SEQ ID NO: __)

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 10 cccagctccagcaatcctgtctgcaatccaggggagaaggtcacaatgacttcagggccagctcaagtgttaattacatgcact
 ggtaccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgc
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 gcggcgggcaacttcccccgacatccagctcctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctgga
 20 ggacgggcagggtcatggacgtggactgtccaccgctctaccacgcaggagggtgagctggcctccacacaaagcgagctca
 cctcagccagaagcactggctgtcagaccgcacctacacctgccagggtcacctatcaaggtcacacctttgaggacagcaccaa
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 acgcagccccgcaagaccaagggctccggcttctctgcttccagccgctggaggtgaccagggccgaatgggagcagaaaga
 tgagttcatctgccgtgcagtccatgaggcagcgagccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgat
 30 aatctaga

2H7 scFv IgE (CH2-CH3-CH4) (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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 FNPPTFGAGTKLELKGGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 5 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSDHVCSDFTP
 PTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQE
 GELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSR
 PSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTV
 10 TSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWP
 GSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLE
 VTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

2H7 scFv MH (SSS) MCH2WTCH3 (nucleotide sequence) (SEQ ID NO: __)

15 aagcttgccgccatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaatgttctct
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 taacccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggtcggcggtggtgatctggaggagtg
 20 ggagctctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggc
 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
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 25 tcctggggggatcgtcagcttctcttcccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtg
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 ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
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 cacaggtgtacacctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcc
 30 cagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgctgctggactccgac
 ggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgatga
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2H7 scFv MH (SSS) MCH2WTCH3 (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILASAPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 5 FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYNSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 10 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
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 PGK

5B9 scFv MTHWTCH2CH3 (nucleotide sequence) (SEQ ID NO: __)

15 aagcttgccgcatgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtatgacgca
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 aaaatctagaactccgctcacgttcggtgctgggaccaagctggagctgaaacgggggtggcggtggtcgggcggtggtgggt
 20 cgggtggcggcggtatgacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgcatcacct
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 30 aaggcttctatcccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtg
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 cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

5B9 scFv MTHWTCH2CH3 (amino acid sequence) (SEQ ID NO: __)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHLSNGITY
 LYWYLQKPGQSPQLLIYQMSNLA SGVPDRFSSSSGSGTDFTLRISRVEAEDVGVYYC
 5 AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
 ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
 SQVFFKMNSLQPNDAIYYCARNGGDNYPIYYYAMDYWGQGTSTVTVSSDQEPKSS
 DKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
 YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
 10 PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
 ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
 LSPGK

Human IgG1 hinge mutations**2H7 scFv- MTH (CSS) WTCH2CH3 (nucleotide sequence) (SEQ ID NO: __)**

15 aagcttgccgccaatggatttcaagtcagatttccagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctct
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 gcagtggtctgggaccttactctctcacaatcagcagagtgaggctgaagatgctgccacttattactgccagcagtgaggattt
 20 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggagggtg
 ggagctctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggc
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 ggtgatacttctacaatcagaagtcaagggcaaggccacactgactgtagacaaatcctccagcagacgctacatgcagctcag
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 25 agggaccacggtcaccgtctcttctgatcaggagcccaaatctgtgacaaaactcacacatccccaccgtccccagcacctgaac
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 ccgcgggaggagcagtagaacagcagctaccgtgtggtcagcgtctcaccgtctgcaccaggactggctgaatggcaagga
 gtacaagtgaaggcttccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
 30 cacaggtgtacaccctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
 cagcgacatcgccgtggagtgaggagcaatgggcagccggagaaacaactacaagaccacgcctcccggtgctggactccgac

ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
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2H7 scFv- MTH (CSS) WTCH2CH3 (amino acid sequence) (SEQ ID NO: __)

5 MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSCDK
10 THTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSGDFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
PGK

15

2H7 scFv- MTH (SCS) WTCH2CH3 (nucleotide sequence) (SEQ ID NO: __)

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25 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcac
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cacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc

cagcgacatcgccgtggagtgaggagagcaatgggcagccggagagaacaactacaagaccacgcctcccgtgctggactccgac
 ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
 ggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaatgatctaga

5 **2H7 scFv- MTH (SCS) WTCH2CH3 (amino acid sequence) (SEQ ID NO: __)**

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHY
 QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 10 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTCPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
 15 SPGK

2H7 scFv- MTH (SSC) WTCH2CH3 (nucleotide sequence) (SEQ ID NO: __)

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 20 ggtaccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagt
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 taaccacccacgcttcggtgctgggaccaagctggagctgaaagatggcggtggtcggcggtggtggatctggaggaggtg
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 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 25 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttactggtacttcgatgtctggggcac
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 gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
 30 ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
 gtacaagtgaagggtctccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac

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 ggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
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5

2H7 scFv- MTH (SSC) WTCH2CH3 (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 10 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 15 NNYKTTTPVLDSGSSFLYSLKLTVDKSRWQQGNVDFSCSVMHEALHNHYTQKSLSL
 SPGK

HIgGMHcys1 (nucleotide sequence) (SEQ ID NO: __)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tg

20

HIgGMHcys2 (nucleotide sequence) (SEQ ID NO: __)

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tct cca ccg tgc

HIgGMHcys3 (nucleotide sequence) (SEQ ID NO: __)

25 gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tcc cca gca cct

HuIgG1 MTCH3Y405 (nucleotide sequence) (SEQ ID NO: __)

gggcagccccgagaaccacaggtgtacacctgccccatcccgaggagatgaccaagaaccaggtcagcctgacctgcct
 ggtaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc

ccgtgctggactccgacggctccttctacctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctctc
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HuIgG1 MTCH3Y405 (amino acid sequence) (SEQ ID NO: __)

5 GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDS DGSFYLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgG1 MTCH3A405 (nucleotide sequence) (SEQ ID NO: __)

gggcagccccgagaaccacaggtgtacaccctgcccccatcccgaggagatgaccaagaaccaggtcagcctgacctgcct
10 ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttcgccctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgG1 MTCH3A405 (amino acid sequence) (SEQ ID NO: __)

15 GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDS DGSFALYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgG1 MTCH3A407 (nucleotide sequence) (SEQ ID NO: __)

Gggcagccccgagaaccacaggtgtacaccctgcccccatcccgaggagatgaccaagaaccaggtcagcctgacctgcc
20 tggtaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcct
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HuIgG1 MTCH3A407 (amino acid sequence) (SEQ ID NO: __)

25 GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDS DGSFFLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgG1 MTCH3Y405A407 (nucleotide sequence) (SEQ ID NO: __)

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
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ccgtgctggactccgacggctccttctacctgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

5

HuIgG1 MTCH3Y405A407 (amino acid sequence) (SEQ ID NO: __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP
VLDS DGSFY LASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

10 **HuIgG1 MTCH3A405A407** (nucleotide sequence) (SEQ ID NO: __)

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
gggtcaaaggcttctatcccagcgacatcgccgtggagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttgcacctgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
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15

HuIgG1 MTCH3A405A407 (amino acid sequence) (SEQ ID NO: __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP
VLDS DGSFALASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

20 **2H7 scFv MTH (SSS) WTCH2MTCH3Y405** (nucleotide sequence) (SEQ ID NO: __)

aagcttgccgccatggatttcaagtgcagatttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctct
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25 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcgggtggtgatctggaggaggtg
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cagcctgacatctgaagactctgcggctctatttctgtgcaagagtgggtgactatagtaactcttactggctactogatgtctggggcac
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tcctggggggaccgtcagttcttcttcccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtg
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 ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
 gtacaagtgaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
 5 cacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcc
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 ggctccttctacctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
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10 **2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (amino acid sequence) (SEQ ID NO: __)**
 MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 15 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALFAPIE
 KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSGSFYLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLS
 20 PGK

2H7 scFv MTH (SSS) WTCH2MTCH3A405 (nucleotide sequence) (SEQ ID NO: __)

aagcttccgccatggatttcaagtcagatttccagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 ccagcttccagcaatctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgaagttacatgcact
 25 ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagt
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 taaccacccacgctcgggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagctggtaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
 tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaat
 30 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcac

agggaccacgggtcaccgtctcttctgatcaggagcccaaactcttctgacaaaactcacacatccccaccgtccccagcacctgaac
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 gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
 ccgcgaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
 5 gtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaac
 cacaggtgtacaccctgccccatccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatcc
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 ggctccttcgccctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcata
 ggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

10

2H7 scFv MTH (SSS) WTCH2MTCH3A405 (nucleotide sequence) (SEQ ID NO: __)

mdfvqifisfllisasviiargqivlsqspailsaspgekvtmtcrassvsymhwyqqkpgsspkpwiypslnlasgvparf
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 kasgytftsynmhwwkqtrpglewigaiypngdtsynqkfkgtatltvdkssstaymqlssltedsavyfcarvvyysn
 15 sywyfdvwtgttvtvssdqepkssdkthtspspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnw
 yvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsreemt
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 slspgk

20 2H7 scFv MTH (SSS) WTCH2MTCH3A407 (nucleotide sequence) (SEQ ID NO: __)

aagcttgcgccatggatttcaagtgacagattttagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
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 ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgttcagt
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 25 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggatctggaggaggtg
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 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcag
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 30 agggaccacgggtcaccgtctcttctgatcaggagcccaaactcttctgacaaaactcacacatccccaccgtccccagcacctgaac
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 cacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggctaaaggcttctatcc
 5 cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtggactccgac
 ggctccttctctcgcagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgatga
 ggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

2H7 scFv MTH (SSS) WTCH2MTCH3A407 (amino acid sequence) (SEQ ID NO: __)

10 MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 15 THTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSDGSFFLASKLTVDKSRWQQGNVFSQSVMHREALHNHYTQKSLSLS
 PGK

20

2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (nucleotide sequence) (SEQ ID NO: __)

aagcttgccgccatggattttcaagtgcagattttcagcttctgctaatacgtgcttcagtcataattgccaggagacaaattgttctct
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 25 ggtaccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtccctgctcgttcagtg
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 30 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
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 5 gtacaagtgaaggctccaacaaagccctcccagcccccatcgagaaaacaatctcaaagccaaagggcagccccgagaac
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10

2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (amino acid sequence) (SEQ ID NO: __)

MDFQVQIFSLLISASVILARGQIVLSQSPAILSPGGEKVTMTCRASSSVSYMHWY
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 15 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 20 KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSDGSFYLAASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
 PGK

2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (nucleotide sequence) (SEQ ID

25 NO: __)

aagcttgcgcgatggatttcaagtcagatttccagcttctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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 30 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtg
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 10 cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
 ggctccttcgccctgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatg
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2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (amino acid sequence) (SEQ ID

15 NO:___)
 MDFQVQIFSLLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 20 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSGFSALASKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLS
 25 PGK

2H7 scFv MTH (SCC) WTCH2CH3 (nucleotide sequence)

aagcttgcgccatggatttcaagtgcagatttgcagttcctgctaatactgcttcagtcataattgccagaggacaaattgttctct
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 30 ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtg
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 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcag
 5 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcac
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 10 gtacaagtgaaggctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
 cacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
 cagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgctgctggactccgac
 ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgcatga
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15

2H7 scFv MTH (SCC) WTCH2CH3 (amino acid sequence)

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 20 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 25 NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
 SPGK

2H7 scFv MTH (CSC) WTCH2CH3 (nucleotide sequence)

aagcttgccgcatggatttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctct
 30 cccagctctcagcaatctgtctgcatctccaggggagaagggtcacaatgacttgagggccagctcaagtgtaagttacatgcact
 ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgc

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 5 ggtgatacttctcacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
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 10 ccgcgaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
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 15 ggctctgcacaaccactacacgcagaagagcctctccctgctccgggtaaatgatctaga

2H7 scFv MTH (CSC) WTCH2CH3 (amino acid sequence)

MDFQVQIFSLLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 20 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
 THTSPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGVVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 25 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 NNYKTTTPVLDSGDSFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
 SPGK

2H7 scFv MTH (CCS) WTCH2CH3 (nucleotide sequence)

aagcttccgccatggatttcaagtgagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 30 ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgaagttacatgcact

ggtaccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagt
 gcagtggtctgggaccttactctctcacaatcagcagagtgaggctgaagatgctgccacttattactgccagcagtgaggatt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctcggggctgagctggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggc
 5 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaat
 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggcttatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcac
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 tcctggggggaccgtcagcttctcttcccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtg
 10 gtggtggacgtgagccacgaagaccctgaggtaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
 ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
 gtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaac
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 cagcgacatcgccgtggagtgggagagcaatgggcagccggagacaactacaagaccacgcctcccgtgctggactccgac
 15 ggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgatga
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2H7 scFv MTH (CCS) WTCH2CH3 (amino acid sequence)

MDFQVQIFSFLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 20 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
 THTCPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 25 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 NNYKTTTPVLDSGFSFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
 SPGK

30 HuIgAHIgA-T4-ORF (nucleotide sequence)

5 tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
 ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
 gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacgtgacctgtgtggctgctacagcgtg
 tccagtgtcctgccgggctgtgccgagccatggaacatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
 10 cgctaaccgccaccctctcaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccc
 tgaacgagctggtgacgtgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggctgcaggggtcacagg
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 ctgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggcccacgaggccctgccgctggccttcac
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 15 ac

HuIgAHIgA-T4-ORF (amino acid sequence)

DQVPVSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
 TFTWTPSSGKSAVQGPDRDLGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
 15 PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQ
 ELPREKYLTWASRQEPSQGTTFFAVTSILRVA AEDWKKGDTFSCMVGHEALPLAF
 TQKTIDRLAGKPTHVNVSVVMAEVDADPSN

1D8-IgAH IgA-T4-CD80 (nucleotide sequence)

20 aagcttatggatttcaagtgcagattttcagcttctgctaatacgtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
 agtctccaacaaccatagctgcacatccaggggagaaggtcacatcacctgccgtgccagctccagtgaagttacatgtactggt
 accagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
 gtgggtctgggacctcttattctctcgcaatcaacacccatggagactgaagatgctgccacttattactgtcagcagtgagtagtact
 ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcggcggtggtgggtcgggtggcgggcg
 25 gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcatgactgtctctgggtt
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 30 ccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtc
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 aaccgccaccctctcaaaatccggaacacattccggcccgaggtccacctgctgccgccgctcgaggagctggccctgaa
 cgagctggtgacgctgacgtgcctggcacgtggcttcagcccaaggatgtgctggtcgtggctgcaggggtcacaggagct
 gccccgcgagaagtacctgacttgggcaccccgagggagccaggggcaccaccaccttcgctgtgaccagcatactgc
 5 gctggcagccgaggactggaagaagggggacaccttctcctgcatggtggccacgaggccctgccgctggccttcacacag
 aagaccatcgaccgcttggcgggtaaaccacccatgcaatgtgtctgttgcacgggaggtggacgcggtccttcgaaca
 cctgctcccatcctgggccattacctaatactcagtaaatagaattttgtgatgtgctgcctgacctactgcttggcccaagatgcag
 agagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgatac

AA

10 **1D8 scFv IgAH IgA-T4-CD80 (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTTTCRASSSVSYMYWY
 QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGITYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 15 INSLQTTDDTAMYYCARIHFDYWGGQVMVTVSSDQVPSTPPTPSPSTPPTPSPSCC
 HPRLSLHRPALEDLLLSEAILTCTLTGLRDASGVTFWTPSSGKSAVQGPPDRDL
 CGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTRPEVHLLPP
 PSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTT
 FAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVM
 20 AEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

human IgE Fc (CH2-CH3-CH4) ORF (nucleotide sequence)

tgatcacgtctgctccagggacttcaccccgcccaccgtgaagatcttacagtcgtcctgcgacggcgggcgggcacttcccccg
 accatccagctcctgtgcctcgtctctgggtacaccccgaggactatcaacatcacctggctggaggacgggcaggtcatggacg
 25 tggacttgtccaccgcctctaccacgcaggaggggtgagctggcctccacaaaagcgagctcacctcagccagaagcactggc
 tgtcagaccgcacctacacctgccagggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattccaacc
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 gacctggcaccagcaaggggaccgtgaacctgacctgggtcccgggccagtggaagcctgtgaaccactccaccagaaagg
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 30 ccagtgagggtgacccacccacacctgccaggggcctcatgcggtccacgaccaagaccagcgcccgcgctgctgccccg
 gaagtctatgcgttgcgacgccggagtgccggggagccgggacaagcgacccctgcctgcctgatccagaactcatgcct

gaggacatctcgggtcagtggtgcacaacgaggtgcagctcccgagcggcgccggcacagcacgacgcagccccgcaagacc
 aagggtccgggtctctcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
 tccatgaggcagcgagccccctcacagaccgtccagcgagcggtgtctgtaaattccggtaaagcgatccttcgaa

AA

5 **human IgE Fc (CH2-CH3-CH4) ORF (amino acid sequence)**

DHVCSDFTPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
 DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
 PRGVSA YLSRSPFDL FIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE
 EKQRNGTLTVTSTLPVGT RDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
 10 VYAFATPEWPGSRDKRTLACLIQNFM PEDISVQWLHNEVQLPDARHSTTQPRKTK
 GSGFFVFSRLEVTRA EWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

ID8 scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)

aagcttatggatttcaagtcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactc
 15 agtctccaacaaccatagctgcattctccaggggagaaggtcaccatcacctgccgtgccagtcagtgtaagttacatgtactgg
 accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
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 20 ctattaaccagcgatggtgtacttgattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
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 25 ttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacctcagccagaagcactggctgtca
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 30 cagggtgacccacccccacctgccaggggccctcatgcgtccacgaccaagaccagcgggcccgctgctgccccggaagtct
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 ggcagcgcagccccctcacagaccgtccagcgagcgggtgtctgtaaaccggtaaagcggtatccttgaagctcccatcctgggc
 cattaccttaatctcagtaaattggaattttgtgatatgctgcctgacctactgctttgcccccaagatgcagagagagaaggagggaatg
 5 agagattgagaagggaaggtgtacgccctgtataaatcgata

1D8-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)

MDFQVQIFSLLISASVMSRGVDIVLTQSPTTIAASPGKEKVTITCRASSSVSYMYWY
 QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 10 SSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 INSLQTDDTAMYICARHFDYWGGQVMVTVSSDHVCSRDFTPPTVKILQSSCDGG
 GHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLS
 QKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSFDFLRKSPTI
 15 TCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTTLVTSTLPVGTRDWI
 EGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLI
 QNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQKDE
 FICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISVNGIFVICCLTYCFAP
 RCRERRRNERLRRESVRPV

20

5B9-IgAH IgA-T4-CD80 (nucleotide sequence)

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 ggctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtcctacatagtaatggcatca
 cttatttgattggtatctgcagaagccaggccagtcctcctcagctcctgattatcagatgtccaaccttgcctcaggagtcccagaca
 25 ggttcagtagcagtggtcaggaactgattcacactgagaatcagcagagtgaggctgaggatgtgggtgtttattactgtgctc
 aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcgggtggctcgggcgggtgggtgggt
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 30 aaaatgaacagtctgcaacctaatgacacagccatttactgtgccagaaatgggggtgataactacccttattactatgctatgga
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 ctgttgtcatggcggaggtggacgcggatccttgaacaacctgctccatcctgggccattaccttaatctcagtaaattgaatttt
 10 gtgalatgctgcctgacctactgctttgcccccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgcc
 ctgtataaatcgatac

5B9-IgAH IgA-T4-CD80 (amino acid sequence)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHNSNGITY
 15 LYWYLQKPGQSPQLLIYQMSNLASGVPDFRFSSSSGSGTDFTLRISRVEAEDVGVYYC
 AQNLELPLIFGAGTKLELKRGGGGSGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
 ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSSGITDYNAAFISRLSITKDDSK
 SQVFFKMNSLQPNDAIYYCARNGGDNYPIYYAMDYWGQGTSTVTVSSDQVPVST
 PPTPSPSTPPTPSPSCCHPRI SLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWPS
 20 SGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLS
 KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKY
 LTWASRQEPSQGTTTFAVTSILRVAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
 LAGKPTHVNVSVVMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRER
 RRNERLRRESVRPV

25

5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)

aagcttgcgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
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 ctattttgtattggtatctgcagaagccaggccagctctcctcagctcctgattatcagatgtccaaccttgcctcaggagtccagaca
 30 ggttcagtagcagtggttcaggaactgatttcacactgagaatcagcagagtgaggctgaggatgtgggtgtttattactgtgctc
 aaaatctagaactccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcgggcgggtgggtgggt

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 aaaatgaacagctctgcaacctaatgacacagccattattactgtgccagaaatgggggtgataactacccttattactatgctatgga
 5 ctactggggtaaggaacctcagtcaccgtctcctctgatcacgtctgtccagggaacttccccgcccaccgtgaagatcttaca
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 10 tccgcaagtcgccacgatcacctgtctgggtggacctggcaccagcaaggggaccgtgaacctgacctgggtccggggcca
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 15 ccggcacagcacgacgcagccccgaagaccaagggctccggcttctcgtcttcagccgcctggagggtgaccaggggccgaat
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20 **5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)**

MRFSQQLLGLLVLPWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHLSNGITY
 LYWYLQKPGQSPQLLIYQMSNLASGVPDFRFSSSSGSGTDFTLRISRVEAEDGVVYYC
 AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSL
 ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVWISGGITDYNAAFISRLSITKDDSK
 25 SQVFFKMNSLQPNDAIYYCARNGGDNYPIYYYAMDYWGQGTSTVTVSSDHVCSR
 DFTPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTAS
 TTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSA
 YLSRPSFDLFIKRSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNG
 TLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATP
 30 EWPGSRDKRTLACLIQNFMPEISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFS
 RLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPWAITLISV
 NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2e12-scFv-IgAH IgA-T4-CD80 (nucleotide sequence)

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
 aatctccagcttctttggctgtgtctctaggtcagagagccacatctcctgcagagccagtgaagtggtgaatattatgtcacaaagt
 5 taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgtgcatccaacgtagaatctggggtcctgcc
 aggttttagtggcagtgggctctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
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 tcgggtggcggcgatctcaggtgcagctgaaggagtcaggacctggcctgggtggcgccctcacagagcctgtccatcacatgc
 accgtctcagggttctcattaaccggctatgggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
 10 atgggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttctt
 aaaaatgaacagctctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggact
 actgggggcaaggaacctcagtcaccgtctcctcagatcagccagttccctcaactccacctaccccatctccctcaactccaccta
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 15 cacctgaccgtgacctctgtggctgctacagcgtgtccagtgctcctgccgggctgtgccgagccatggaacctgggaagaccttc
 acttgcactgctgcctaccccgagtccaagaccccgctaaccgccacctctcaaatccggaaacacattccggcccgagggtcc
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 ccaggggcaccaccaccttcgtgtgaccagcactatgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcac
 20 ggtggggccacgaggccctgccgtggccttcacacagaagaccatcgaccgcttggcgggtaaaccacccatgtcaatgtgtct
 gttgtcatggcggaggtggacgcggatccttcgaacaacctgctccatcctgggccattaccttaatctcagtaaatggaattttgt
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 gtataaatcgatac

25 2e12-scFv-IgAH IgA-T4-CD80 (amino acid sequence)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYP
 CQQSRKVPWTFGGGKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSL
 ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMWGDGSTDYNSALKSRLSITKD
 30 NSKSQVFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQGTSVTVSSDQPVPS
 TPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTP
 SSGKSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLS

KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKY
 LTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
 LAGKPTHVNVSVVMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRER
 RRNERLRRESVRPV

5

2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)

aagcttatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc
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 10 aggttttagtggcagtggtgtgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
 aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtggg
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 15 aaaaatgaacagctctgcaaactgatgacacagccagatactactgtccagagatgggtatagtaactttcattactatgttatggact
 actggggtcaaggaacctcagtcaccgtctcctcagatcacgtctgtccagggacttcaccccgccaccgtgaagatcttacag
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 cacctgggtggaggacgggcaggtcatggacgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacac
 aaagcgagctcacctcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttga
 20 ggacagcaccaagaagtgtgcagattccaacccgagaggggtgagcgctacctaagccggcccagcccgttcgacctgttcat
 ccgcaagtcgcccacgatcacctgtctggtgggtggacctggcaccagcaaggggaccgtgaacctgacctggtccggggcca
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 25 gcacctcgcctgcctgatccagaacttcagcctgaggacatctcggtgcagtggtgcacaacgaggtgcagctcccgagcgc
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 actgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgata

30

2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)

MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
 CQQSRKVPWTFGGGKLEIKRGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
 ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
 5 KSQVFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQGTSTVTVSSDHVCSR
 DFTPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTAS
 TTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSA
 YLSRPSPFDLFIRKSPTTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNG
 TLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATP
 10 EWPGSRDKRTLACLIQNFMPEISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFS
 RLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPWAITLISV
 NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

500A2 scFv (nucleotide sequence)

15 atgttgatacatctcagctccttgggcttttactcttctggatttcagcctccagaagtacatagtgctgactcagactccagccactc
 tgtctctaattcctggagaaagagtcacaatgacctgtaagaccagtcagaatattggcacaatctacactggatcaccaaaaacc
 aaaggaggctccaagggtctcatcaagtatgcttcgcagtcacatctctgggatcccctccagattcagtggcagtggttcggaaac
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 20 gctgcagcagtcggttctgaactagggaaacctggggcctcagtgaaactgtcctgcaagacttcaggctacatattcacagatc
 actatatttcttgggtgaaacagaagcctggagaaagcctgcagtgataggaaatgtttatggtggaaatggtgtacaagctaca
 atcaaaaattccagggaaggccacactgactgtagataaaatctctagcacagcctacatggaactcagcagcctgacatctgag
 gattctgccatctattactgtgcaagaaggccggtagcgacgggcatgctatggactactggggtcaggggatccaagttaccgt
 ctctctgatc

25

500A2 scFv (amino acid sequence)

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYH
 QKPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSPWPV
 TFGPGTKLEIKRGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIF
 30 TDHYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMEL
 SSLTSEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSD

NT

5' oligo:

Name : IgGWT3

GTTGTTTTTCGAAGGATCCGCTTTACCCGGAGACAGGGAGAGGCTCTT

5 NT

3' oligo:

Name : hIgGWT5

GTTGTTAGATCTGGAGCCCAAATCTTGTGACAAAACCTCACACATG

NT

10 5' oligo:

Name : FADD5

Sequence

GTTGTGGATCCTTCGAACCCGTTCTTGGTGCTGCTGCACTCGGTGTCG

NT

15 3' oligo:

Name : FADD3

Sequence

GTTGTTATCGATCTCGAGTTATCAGGACGCTTCGGAGGTAGATGCGTC

NT

20 **FADD-CSSCFV (nucleotide sequence)**

gtggatccttcgaacccgttcctggtgctgctgcactcgggtgctccagcctgtcgagcagcgagctgaccgagctcaagttccta
tgcctcgggcgcggtgggcaagcgcaagctggagcgcgtgcagagcggcctagacctcttccatgctgctggagcagaacga
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tgtccccgatgtcatggaactcagacgcatctacctccgaagcgtcctgataactcgagatcgataacaac

30 **FADD-CSSCFV (amino acid sequence)**

VDPSNPFLVLLHSVSSSLSSSELTELKFLCLGRVGKRKLERVQSGLDLFSMLLEQND
LEPGHTELLRELLASLRHDLLRRVDDFEAGAAAGAAPGEEDLCAAFNVICDNVG
KDWRRLARQLKVSDTKIDSIEDRYPRNLTERVRESLRIWKNTOKENATVAHLVGA
LRSCQMNLVADLVQEQARDLONRSGAMSPMSWNSDASTSEAS

5

HCD28tm5B (nucleotide sequence)

GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGTGGTTGGTGTCTGGCTTGCTAT
AGCTTG

10 **HCD28tm3S** (nucleotide sequence)

GTTGTTTCGAACCCAGAAAATAATAAAGGCCACTGTTACTAGCAAGCTATAGC
AAGCCAG

HCD28tm5' (nucleotide sequence)

15 GTGTGGATCCTCCCTTTTGGGTGCTGGTGGT

HCD28tm3' (nucleotide sequence)

GTTGTTTCGAACCCAGAAAATAATAAAGGCCAC

20 HCD80tm5' (nucleotide sequence)

GTTGTGGATCCTCCTGCTCCCATCCTGG

HCD80tm3' (nucleotide sequence)

25 GTTGTTTCGAACGGCAAAGCAGTAGGTCAGGC

MFADD5BB (nucleotide sequence)

GTTGTGGATCCTTCGAACCCATTCTGGTGCTGCTGCACTCGCTG

MFADD3XC (nucleotide sequence)

GTTGTTATCGATCTCGAGTCAGGGTGTCTGAGGAAGACAC

- 5 **Murine FADD nucleotide sequence** (full length, but without flanking -Ig or transmembrane sequences) (nucleotide sequence)

gtggatccttgaacatggacccattcctgggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatctgatggagctcaa
 gttcttgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgttcacggtgctgctggagca
 gaacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggcctcgtgcgccgacacgatctactgcagcgcctgg
 10 acgacttcgaggcggggacggcgaccgctgcgccccgggggaggcagatctgcaggtggcatttgacattgtgtgacaatg
 tggggagagactggaaaagactggcccgcgagctgaaggtgtctgaggccaagatggatgggattgaggagaagtacccccg
 aagtctgagtgagcgggtaaggagagctgaaagtctggaagaatgctgagaagaagaacgcctcgggtggccggactggtca
 aggcgctgcggacctgcaggctgaatctggtggctgacctggtggaagaagcccaggaatctgtgagcaagagtgagaatatgt
 ccccgactactaagggttcaactgtgtcttctcagaaacaccctgactcgagatcgat

15

Murine FADD (amino acid sequence)

VDPSNMDPFLVLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDLFTVLLE
 QNDLERGHTGLLRELLASLRRHDLQLRLDDFEAGTATAAPPGEADLQVAFDIVCD
 NVGRDWKRLARELKVSEAKMDGIEEKYPRSLSERVRESLKVWKNAEKKNASVA
 20 GLVKALRTCRLNLVADLVEEAQESVSKSENMSPVLRDSTVSSSETP

MCASP3-5 (nucleotide sequence)

GTTGTGGATCCTTCGAACATGGAGAACAACAAAACCTCAGTGGATTCA

- 25 **MCASP3-3 (nucleotide sequence)**

GTTGTTATCGATCTCGAGCTAGTGATAAAAGTACAGTTCTTTCGT

MCASP8-5 (nucleotide sequence)

GTTGTTTCGAACATGGATTTCAGAGTTGTCTTTATGCTATTGCTG

MCASP8-3 (nucleotide sequence)

GTTGTTATCGATCTCGAGTCATTAGGGAGGGAAGAAGAGCTTCTTCCG

5 hcas3-5(nucleotide sequence)

GTTGTGGATCCTTCGAACATGGAGAACACTGAAAACCTCAGTGGAT

hcas3-3 (nucleotide sequence)

GTTGTTATCGATCTCGAGTTAGTGATAAAAATAGAGTTCTTTTGTGAG

10

hcas8-5 (nucleotide sequence)

GTTGTGGATCCTTCGAACATGGACTTCAGCAGAAATCTTTATGAT

hcas8-3 (nucleotide sequence)

15 GTTGTTATCGATGCATGCTCAATCAGAAGGGAAGACAAGTTTTTTTCT

1. 2H7 scFv with alternative VHL11 mutations:**Nucleotide sequence**

20 Aagcttgccgcatgatttcaagtcagattttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctc
 tcccagctctccagcaatctgtctgcatctccaggggagaaggcacaatactgcttcagggccagctcaagtgttaagttacatgcac
 tggtagcagcagaagccaggatcctcccccacccctggattatgccccatccaacctggcttctggagtcctgctcgttcagt
 ggcagtggtctgggaccttactctctcacaatcagcagagtgaggctgaagatgctgccacttattactgccagcagtgaggat
 ttaaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggt
 gggagctctcaggcttatctacagcagctctggggctgag (one of the following: tcn, acn, gan, can, aan,
 25 **cg**n, agn)
 gtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacattaccagttacaatatgactgggtaaagcagaca
 cctagacagggcctggaatggattggagctattatccaggaaatggtgatacttctacaatcagaagttcaagggaaggccac
 actgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcggtctatttctgtgcaag
 30 agtggtgtactatagtaactcttactggtacttcgatgtctggggcacagggaaccacggtcaccgtctctctgatcag

Amino acid sequence

MDFQVQIFSLLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAE (one of the following:
 35 **S, T, D, E, Q, N, R, K, H)**
 VRPGASVKMSCKASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFK

GKATLTVDKSSSTAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVT
VSSDQ

2. VHL11 deletion

5 Nucleotide sequence:

Aagcttgcgccaatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctc
tcccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagttacatgcac
tggtaccagcagaagccaggatcctccccc aaacctggattatgccccatccaacctggcttctggagtcctctgctcgttcagt
ggcagtggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggat
10 ttaaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggt
gggagctctcaggcttatctacagcagctctggggctgaggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggt
acacattaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctattatccaggaaatg
gtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagtcagc
agcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgtactatagtaactcttactgtacttcgatgtctggggcaca
15 gggaccacggtcaccgtctctctgatcag

Amino acid sequence:

MDFQVQIFSFLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWYAPS NLASGV PARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
20 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAEVRPGASVKMSCKA
SGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSST
AYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQ

3. 2H7 VL L106 with alternative mutations

25 Nucleotide sequence:

aagcttgcgccaatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctc
cccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagttacatgcact
ggtaccagcagaagccaggatcctccccc aaacctggattatgccccatccaacctggcttctggagtcctctgctcgttcagt
gcagtggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggatt
30 taaccacccacgttcggtgctgggaccaagctggag (tcn, agn, aan, cgn, can, gan, and non-natural
derivatives of these codons) aaagatggcggtggctcgggcggtggtggatctggaggaggtgggagctc

Amino acid sequence:

MDFQVQIFSFLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
35 QQKPGSSPKPWYAPS NLASGV PARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLE (S, T, R, K, H, Q, N, D, E, and non-natural derivatives of these
amino acids at position 106)KDGGGSGGGGSGGGGSS

4. VL L106 deletion

40 Nucleotide sequence:

Aagcttgcgccaatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctc
tcccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagttacatgcac
tggtaccagcagaagccaggatcctccccc aaacctggattatgccccatccaacctggcttctggagtcctctgctcgttcagt
ggcagtggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggat
45 ttaaccacccacgttcggtgctgggaccaagctggagaaagatggcggtggctcgggcggtggtggatctggaggaggtgg
gagctc

Amino acid sequence:

MDFQVQIFSLLISASVILARGQIVLSQSPAILSPGKVTMTTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLEKDGGGSGGGGSGGGSS

5. IgE CH3 CH4

Nucleotide sequence:

tccaacccgagaggggtgagcgctacctaagccggcccagcccgttcacctgttcacccgaagtcgcccacgatcacctgtc
 tgggtggtggacctggcaccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccacc
 agaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccacctgcccgtgggcacccgagactggatcgaggggg
 10 agacctaccagtgcagggtagccacccccacctgccaggggccctcatgcggtccacgaccaagaccagcgggccgcgtgct
 gccccggaagtctatgcgttgcgacggcgagtgccggggagccgggacaagcgaccctcgctgctgatccagaactt
 catgctgaggacatctcggtgcagtggctgcacaacgaggtgcagctccggacggcggcacagcacgacgcagccccgc
 aagaccaagggtccggcttcttctgcttcagccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgcc
 gtgcagtcctagggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgataatctagaa

Amino acid sequence:

SNPRGVSAAYLSRPSFDLFIRKSPTTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTR
 KEEKQRNGTLTVTSTLPVGTDRDIEGETYQCRVTHPHLPRALMRSTTKTSGPRAA
 PEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRK
 20 TKGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

6. hIgG1H/IgE WCH3 WCH4

Nucleotide sequence:

tgatcaggagcccaaatctctgacaaaactcacacatccccacgtccccagcatccaacccgagaggggtgagcgctaccta
 25 agccggcccagccgttcgacctgttcacccgaagtcgcccacgatcacctgtctggtggtggacclggcaccagcaagggg
 accgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaaggaggagaagcagcgcaatggca
 cgtaaacgtcacgtccacctgcccgtgggcacccgagactggatcgagggggagacctaccagtgcagggtagccacccc
 cacctgccaggggccctcatgcggtccacgaccaagaccagcgggccgcgtgctgccccggaagtctatgcgttgcgagcc
 ggagtggccggggagccgggacaagcgacccctgcctgctgatccagaactcatgctgaggacatctcggtgcagtggct
 30 gcacaacgaggtgcagctccggacggcggcacagcacgacgagccccgaagaccaagggtccggcttcttctgcttca
 gccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctagggcagcgagcccctca
 cagaccgtccagcgagcggtgtctgtaaatcccggtaaatgataatctagaa

Amino acid sequence:

DQEPKSSDKTHTSPSPASNPRGVSAAYLSRPSFDLFIRKSPTTITCLVVDLAPSKGTV
 35 NLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTDRDIEGETYQCRVTHPHL
 PRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLH
 NEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQ
 40 VQRAVSVNPGK

7. IgE WCH2 WCH3 WCH4

Nucleotide sequence:

Tgatcacgtctgtccagggacttcacccgcccacgtgaagatcttacagtcgtctgcgacggcgggcggcacttccccccg
 accatccagctcctgtgctcgtctctgggtacacccagggactatcaacatcacctggctggaggacgggcaggtcatggacg
 45 tggactgtccaccgccttaccacgcaggaggtgagctggcctccacaaaagcgagctcacctcagccagaagcactggc
 tgtcagaccgcacctacactgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattccaaccc
 gagaggggtgagcgctacctaagccggcccagcccgttcgacctgttcacccgaagtcgcccacgatcacctgtctggtggtg
 gacctggcaccagcaagggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaagg
 aggagaagcagcgcaatggcacgttaaccgtcacgtccacctgcccgtgggcacccgagactggatcgagggggagacctta
 ccagtgcagggtagccacccccacctgccaggggccctcatgcggtccacgaccaagaccagcgggccgcgtgctgccccg
 50 gaagtctatgcgttgcgacggcgagtgccggggagccgggacaagcgacccctgcctgctgatccagaactcatgct

gaggacatctcgggtgcagtggtgcacaacgaggtgcagctcccgagcggccggcacagcacgacgcagccccgcaagacc
aagggtccggcttctcgtctcagccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
tccatgaggcagcagccccctcacagaccgtccagcgagcgggtgtctgtaaatcccggtaaatgataatctaga

5 Amino acid sequence:

DHVCSDFTPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
DLSTASTTQEGELASTQSELTLQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
PRGVSA YLSRSPFDL FIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE
EKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
10 VYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
GSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

8. **hIgG1H/IgE CH3 CH4 (ORF)**

Nucleotide sequence:

15 tgatcaggagcccaaatctctgacaaaactcacacatccccaccgtccccagcatccaacccgagaggggtgagcgccctaccta
agccggccagcccggttcgacctgtcatccgcaagtcgcccacgatcacctgtctggtggtggacctggcaccagcaagggg
accgtgaacctgacctggtcccgggagcagtggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcaatggca
cgtaaacctgacgtccacacctgcccgtgggacccgagactggatcgagggggagacctaccagtgcagggtgaccacccc
cacctgcccagggccctcatcggtccacgaccaagaccagcgcccgctgctgccccggaagtctatcggttgcgacgcc
20 ggagtgccggggagccgggacaagcgaccctgcctgcctgatccagaactcatgcctgaggacatctcggtgcagtggtct
gcacaacgaggtgcagctcccgagcccgccacagcacgacgcagccccgcaagaccaagggtccggcttctcgtcttca
gccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctatgaggcagcgagccctca
cagaccgtccagcgagcgggtgtctgtaaatcccggtaaagcgatcctcgaa

25 Amino acid sequence:

DQEPKSSDKTHTSPSPASNPRGVSA YLSRSPFDL FIRKSPTITCLVVDLAPSKGTV
NLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHL
PRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLH
NEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQT
30 VQRAVSVNPGKSGSFE

9. **2H7 VHL11S scFv hIgG1(SSS-S)H hIgE WCH3 WCH4**

Nucleotide sequence:

35 aagcttgccgcatggatttcaagtcagatttccagcttctgtaaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagcttccagcaatctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgaagtacatgcact
ggtaccagcagaagccaggatctccccaaacctggatttatgccccatcaacctggcttctggagtcctgtctgcttcagtg
gcagtggtgtggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtt
taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggtatctggaggaggtg
ggagctctcaggcttactacagcagctctggggtgagtcggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggc
40 tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctcggtctatttctgtcaagagtggtgtactatagtaacttactggtacttcgatgtctggggcac
agggaccacgggtcaccgtcttctgatcaggagcccaatcttctgacaaaactcacacatccccaccgtcctcagcatccaacc
cgagaggggtgagcgccctacctaagccggccagccggttcgacctgttcacccgcaagtcgcccacgatcacctgtctggtggt
45 ggacctggcaccagcaaggggaccgtgaacctgacctgggtccggggcagtgagggaagcctgtgaaccactccaccagaaag
gaggagaagcagcgcaatggcacgttaaccgtcacgtccacctgcccgggtgggacccgagactggatcgagggggagacct
accagtgcagggtgaccacccccacctgcccagggccctcatcggtccacgaccaagaccagcgggccgctgctgcccc
ggaggtctatcggttgcgacgccggagtggccggggagccgggacaagcgaccctcgctgctgatccagaacttcagcc
tgaggacatctcggtgcagtggtgcacaacgaggtgcagctcccgagcggcgccagcagcagcagcagccccgcaagacc
50 aagggtccggcttctcgtctcagccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
tccatgaggcagcgagccccctcacagaccgtccagcgagcgggtgtctgtaaatcccggtaaatgataatctaga

Amino acid sequence:

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 5 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSSASNPRGVSA YLSRPSFDLFIKRSPTITCLVVDLAPSKGTVNLTWSRASG
 KPVNHSTRKEEKQRNGTLTVTSTLPVGT RDWIEGETYQCRVTHPHLPRALMRSTT
 10 KTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPE DISVQWLHNEVQLPDAR
 HSTTQPRKTKGSGFFVFSRLEVTRA EWEQKDEFICRAVHEAASPSQTVQRAVSVNP
 GK

10. 2H7 VHL11S scFv hIgG1(SSS-P)H hIgE WCH3 WCH4

Nucleotide sequence:

aagcttgccgcatggatttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 ccagctctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcact
 ggtaccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtg
 20 gcagtggtctctgggacctcttactctctcacaatcagcagagtgaggctgaagatgctgccacttactactgccagcagtgaggattt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgtctgcaaggcttctggc
 tacacattaccagttacaatatgactgggtaagcagacacctagacaggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaaggccaaggccacactgactgtagacaaatcctccagcacagcctacalgcagctcag
 25 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcac
 agggaccacgggtaccgtctctctgatcaggagccc aaatctctgacaaaactcacacatccccaccgtcccc agcatccaacc
 cgagaggggtgagcgctacctaagccggcccagccggttcacctgttcacccgcaagtcgccacgatcacctgtctggtggt
 ggacctggcaccacgaaggggaccgtgaacctgacctggtcccggccagtggggaagcctgtgaaccactccacagaaag
 gaggagaagcagcgcaatggcacgttaacctgcacgtccaccctgccggtgggacccgagactggatcgagggggagacct
 30 accagtgacgggtgacccacccccacctgccaggggccctcatgcggtccacgaccaagaccagcggcccgctgctgcccc
 ggaagtctatgcgttgcgacgccggagtggtggcggggagccgggacaagcgcaccctcgctgctgatccagaacttcatgcc
 tgaggacatctcgggtgcagtggtgcacaacgaggtgcagctccggagcccggcacagcacgacgcagccccgcaagacc
 aagggtccggcttctctcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
 tccatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaatgataatctaga
 35

Amino acid sequence:

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 40 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSPASNPRGVSA YLSRPSFDLFIKRSPTITCLVVDLAPSKGTVNLTWSRASG
 KPVNHSTRKEEKQRNGTLTVTSTLPVGT RDWIEGETYQCRVTHPHLPRALMRSTT
 KTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPE DISVQWLHNEVQLPDAR
 45 HSTTQPRKTKGSGFFVFSRLEVTRA EWEQKDEFICRAVHEAASPSQTVQRAVSVNP
 GK

10. 2H7 VL L106S

aagcttgccgcatggatttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 50 ccagctctccagcaatcctgtctgcattctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcact
 ggtaccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtg

gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtg
ggagctc

5 Amino acid sequence:

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLESKDGGGSGGGGSGGGGSS

10 11. 2H7 VL L106S scFv

Nucleotide sequence:

aagcttccgccatggatttcaagtgcagatttccagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctc
cccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagttacatgcact
ggtagcagcagaagccaggtatcctcccccaccctggatttgcacatccaacctggcttctggagtcctgctcgttcagtg
15 gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taaccacccacgttcggtgctgggaccaagctggagcttaagatggcgggtggctcgggcgggtggatctggaggaggtg
ggagctctcaggcttatctacagcagctctggggctgagctggtaggcctggggcctcagtggaatgtctgcaaggcttctggc
tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaat
ggtagacttcttacaatcagaagttcaaggccaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
20 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtagctcgtctggtgggc
agggaccacgggtaccgtctcttctgatcag

Amino acid sequence:

25 MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLESKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQ

30 12. 2H7 scFv VL L106S VHL11S scFv

Nucleotide sequence:

Aagcttccgccatggatttcaagtgcagatttccagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctc
tcccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagttacatgcac
tggtaccagcagaagccaggtatcctcccccaccctggatttgcacatccaacctggcttctggagtcctgctcgttcagtg
35 ggcagtggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
tttaaccacccacgttcggtgctgggaccaagctggagcttaagatggcgggtggctcgggcgggtggatctggaggaggt
gggagctctcaggcttatctacagcagctctggggctgagctggtaggcctggggcctcagtggaatgtctgcaaggcttctg
gctacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaa
atggtagacttcttacaatcagaagttcaaggccaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctc
40 agcagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtagctcgtctggtgggc
acagggaccacgggtaccgtctcttctgatcag

Amino acid sequence:

45 MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLESKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQ

50 10. Human IgD hinge linker with attached restriction sites

Nucleotide:

gtggatccaggttcgaagtctccaaaggcacaggcctcctccgtgccactgcacaacccaagcagagggcagcctcgccaa
ggcaaccacagccccagccaccaccgtaacacaggaagaggaggagaagagaagaagaaggagaaggagaagaggaa
caagaagagagagagacaaagaccgggtgcagtcgacg

5 Amino acid:

VDPGSKSPKAQASSVPTAQPPAEGSLAKATTAPATTRNTGRGGEEKKKKEKEKEEQ
EERETKTGAVD

Sequence of Native IgD hinge domain:

10 (includes a cysteine residue—we truncated the hinge prior to that residue for these
constructs:)

Nucleotide:

gagtcctccaaaggcacaggcctcctccgtgccactgcacaacccaagcagagggcagcctcgccaaggcaaccacagccc
cagccaccaccgtaacacaggaagaggaggagaagaagaaggagaaggaagaagaagagagagaga
15 gacaaagacaccagagtgtccgagccacaccagcctctggcgtctacctgtaaccct

Amino acid sequence:

ESPKAQASSVPTAQPPAEGSLAKATTAPATTRNTGRGGEEKKKKEKEKEEQEERET
KTPECPSHTQPLGVYLLTP

20

12. 2H7 VH L11S

Nucleotide sequence:

caggcttatctacagcagtcgtggggctgagtcggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacatt
accagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact
25 tctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcagcctacatgcagctcagcagcctga
catctgaagactctgcggctctattctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagagacc
acggtcaccgtctctct

Amino acid sequence:

30 QAYLQQSGAESVRPGASVKMSCKASGYTFTSYNMHWVKQTPRQGLEWIGATYPG
NGDTSYNQKFKGKATLTVDKSSSTAYMQLSSLTSEDSAVYFCARVVYYSNSYWY
FDVWGTGTTVTVSS

13. 2H7 VH L11S scFv

35 Nucleotide sequence:

aagcttgccgcatggaatttcaagtcagatttcagcttctgtaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctgatttatgccccatccaacctggcttctggagtcctgctcgttcagtg
gcagtggtgtgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggatt
40 taaccacccacggtcgtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggtggtggtggtggtg
ggagctctcaggttatctacagcagtcgtggggctgagtcggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggc
tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcagcctacatgcagctcag
cagcctgacatctgaagactctgcggctctattctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcac
45 agggaccacgggtcaccgtctcttctgacag

Amino acid sequence:

50 MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK

ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFVDVWGTGTTVTVSSDQ

14. 2H7 scFv VH L11S hIgG1 (CSC-S)H WCH2 WCH3

5 Nucleotide sequence:

aagcttgcgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
cccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcact
ggtaccagcagaagccaggatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
gcagtggtgtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggttt
10 taaccacccacgttcggtgtctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagctctggggctgagctgtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggct
acacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaatg
gtgatacttctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagc
agcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcaca
15 gggaccacgggtcaccgtctcttctgatcaggagcccaaatctgtgacaaaactcacacatctccaccgtgctcagcacctgaactc
ctgggtggaccgtcagcttctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggt
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cgcgaggagagcagtagaacagcagctaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggtgaatggcaaggag
tacaagtgaaggcttccaacaaagccctccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaacc
20 acaggtgtacacctgccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcca
agcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccggtgctggactccgacg
gtccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaatgatctaga

25 Amino acid sequence:

MDFQVQIFSLLISASVUARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
30 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFVDVWGTGTTVTVSSDQEPKSCDK
THTSPPCSAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSGFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL
35 SPGK

15. 2H7 scFv VH L11S IgE WCH2 WCH3 WCH4

Nucleotide sequence:

aagcttgcgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
40 cccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcact
ggtaccagcagaagccaggatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
gcagtggtgtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggttt
taaccacccacgttcggtgtctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagctctggggctgagctgtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggct
45 acacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaatg
gtgatacttctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagc
agcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcaca
gggaccacgggtcaccgtcttctgatcacgtctgtccagggacttcacccgccaccgtgaagatcttacagtcgtctcgac
ggcgggcgggcacttcccccgaccatccagctcctgtgctcgtctctgggtacacccagggactatcaacatcacctggctgg
50 aggacgggcaggtcatggagctggactgtccaccgcctctaccacgcaggaggtgagctggcctccacacaaagcgagctc
acctcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggctcacacctttgaggacagcacca

agaagtgtgcagattccaacccgagaggggtgagcgcctacctaagccggcccaagcccgttcgacctgttcacccgcaagtcgc
 ccacgatcacctgtctgtgtggacctggcaccagcaaggggaccgtgaacctgacctggccccgggagtgaggagccct
 gtgaaccactccaccagaaaggaggagaagcagcgcgaatggcacgttaacctgacgtccaccctgccgggtgggcacccgag
 actggatcgagggggagacctaccagtgcagggtagccacccccacctgccagggccctcatgagggtccacgaccaagac
 5 cagcggcccgctgctgccccggaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcc
 tgctgatccagaactcatgcctgaggacatctcggtagtgagtgacacaacgaggtgcagctccggagccccggcacagc
 acgacgcagccccgcaagaccaagggctccggcttctcgtcttcagccgctggaggtgaccagggccgaatgggagcagaa
 agatgagttcatctgccgtgcagtcctgaggcagcagccctcacagaccgtccagcgagcgggtgtctgtaaatcccggtaaa
 tgataatctaga

10

Amino acid sequence:

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 15 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYNSYWFYFDVWGTGTTVTVSSDHVCSRDF
 PPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQ
 EGELASTQSELTLQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLS
 RPSPFDLFIKRSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLT
 20 VTSTLPVGTTRDWIEGETYQCRVTHPLPRALMRSTTKTSGPRAAPEVYAFATPEW
 PGSRDKRTLACLIQNFMPEISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRL
 EVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

16. 2H7 scFv VH L11S mIgE WCH2 WCH3 WCH4

25 Nucleotide sequence:

aagcttgcgcgcattggaatttcaagtgagatttgcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacatgactgcagggccagctcaagtgttaagtacatgcact
 ggtaccagcagaagccaggtacctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
 gcagtggtctgggaccttactcttcacaatcagcagagtgaggctgaagatgctgccacttactgccagcagtgaggattt
 30 taaccacccacgcttgcgtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagcttggggctgagtgctgtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggct
 acacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaatg
 gtgatacttctacaatcagaagttcaagggaagccacactgactgtagacaaatcctccagcacagcctacatgcagctcagc
 agcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcaca
 35 gggaccacggtcaccgtctcttctgacaggttcacgtgcaacatcactgagccacacctggagctactccattatcctgcgacc
 ccaatgcattccactccaccatccagctgtactgcttattatggccacatcctaaatgatgtctctgtcagctggctaatggacgatc
 gggagataactgatacttgcacaaactgttctaataaggagggaaggcaaaactagcctctacctgcagtaaaactcaacatcactg
 agcagcaatggatgtctgaaagcaccttcacctgcaaggtcacctcccaaggcgtagactattggcccacactcggagatgccca
 gatcatgagccacggggtgtgattacctacctgatccacccagccccctggacctgtatcaaaacggtgctcccaagcttacctgt
 40 ctggtggtggacctggaaagcgagaagaatgtcaatgtgacgtggaaccaagagaagaagacttcagtcacatcccagtggt
 acactaagcaccacaataacgccacaactagtatccctccatcctgctgtagttgccaaggactggattgaaggctacggctatc
 agtgcatagtggaccacctgattttcccaagccattgtgcgttccatccaagacccagccagcgctcagcccccgaggta
 tatgtgtccaccaccagaggaggagcgcaggacaaacgcacactcacctgtttgatccagaacttcttccctgaggatatctct
 gtgcagtggtgggggatggcaaaactgatctcaaacagccagcacagtagcacacacccctgaaatccaatggctccaatcaa
 45 ggcttcttcatcttcagtcgcttagaggtcgccaagacactctggacacagagaaaacagttcacctgccaagtgtatcatgaggc
 acttcagaaaccaggaactggagaaaacaatatccacaagccttgtaaacacctcctccgtccatcctagtaatatctagag

Amino acid sequence:

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 50 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK

ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDHVRPVNIT
 EPTLELLHSSCDPNAFHSTIQLYCFIYGHILNDVSVSWLMDDREITDTLAQTVLKE
 EGKLASTCSKLNITEQQWMSESTFTCKVTSQGV DYLAHTRRCPDHEPRGVITYLIP
 5 PSPLDLYQNGAPKLTCLVVDLESEKNVNVNTWNQEKKTSVSASQWYTKHHNNATT
 SITSILPVVAKDWIEGYGYQCIVDHPDFPKPIVRSITKTPGQRSAPENVYVFPPEESEE
 DKRTLTLCLIQNFFPEDISVQWLGDGKLISNSQHSTTTPLKSNGSNQGFFIFSRLEVAK
 TLWTQRKQFTCQVIHEALQKPRKLEKTISTSLGNTSLRPS

10 **17. 2H7 scFv VH L11S hIgA WH WCH2 T4CH3**

Nucleotide sequence:

aagcttgcgccatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcact
 ggtaccagcagaagccaggatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttccagtg
 15 gcagtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggatt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtgatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagctgtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggct
 acacatttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatg
 gtgatacttctacaatcagaagtcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagc
 20 agcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcaca
 gggaccacgggtaccgtctctctgcatcagccagttccctcaactccacctacccatctcctcaactccacctacccatctccct
 catgctgccacccccgactgtcactgcaccgaccggcctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactg
 accggcctgagagatgcctcaggtgtcaccttcacctggagccctcaagtgggaagagcgctgttcaaggaccactgaccgtg
 acctctgtggctgctacagcgtgtccagigtctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgct
 25 gctacccccgagtccaagaccccgtaaacgccacctctcaaatccggaacacattccggcccagggtccacctgtgccc
 ccgcccgtcggaggagctggccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagcccaaggatgtgctggtt
 cgctggctgcaggggtcacaggagctgccccgcgagaagtacgtgactgggcatcccgaggagcccagccagggcacca
 ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcattggtggccacg
 agggcctgcccgtggccttcacacagaagaccatcgaccgctggcggttaaaccacccatgtcaatgtgtctgttgcattggcg
 30 gaggtggactgataatctaga

Amino acid sequence:

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 35 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQVPVSTPPT
 PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWTPSSG
 KSAVQGPPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
 40 GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLT
 WASRQEPSQGTTTFAVTSILRVA AEDWKKGDTFSCMGHEALPLAFTQKTIDRLA
 GKPTHVNVSVVMAEVD

18. 2H7 scFv VH L11S mIgA WH WCH2 T4 CH3

Nucleotide sequence:

aagcttgcgccatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcact
 ggtaccagcagaagccaggatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttccagtg
 50 gcagtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggatt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtgatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagctgtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggct

acacatttaccaggttacaatatgcactgggttaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatg
 gtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagc
 agcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctgggcaca
 5 gggaccacgggtaccgtctcttctgatcacatctgttctccttactactcctcctccaccttctgccagcccagcctgtcactgca
 gggccagctcttgaggacctgtcctgggttcagatgccagcatcacatgtactctgaatggcctgagagatcctgagggagctg
 tcttccctgggagccctccactgggaaggatgcagtgacagaagaaagctgtgcagaattcctgcggctgctacagtgtgccagc
 gtctgtcctggctgtgtgagcgtggaacagtggcgcatcattcaagtgcacagttaccatcctgagctgtacaccttaactggc
 acaattgccaaagtcacagtgaacaccttcccaccccaggtccacctgtaccgccgccgtcggaggagctggccctgaatgag
 10 ctctgtccttgacatgcctgggtgcgagcttcaaccctaaagaagtgtggtgcgatggctgcagtgaaatgaggagctgtcccc
 agaaagctacctagtgtttgagcccctaaaggagccaggcgaggagccaccacctacctggtgacaagcgtgttgcgtgtatca
 gctgaaatctggaacagggtgaccagtactcctgcaggtgggccacgaggccttgcccatgaacttcaccacagaagaccatcg
 accgtctgtcgggttaaaccaccaatgtcagcgtgtctgtgatcatgtcagaggagattgataatctagat

Amino acid sequence:

15 MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTISYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDHICSPPTTP
 20 PPPSCQPSLSLQRPALDLLLLGSDASITCTLNGLRDPEGAVFTWEPSTGKDAVQKK
 AVQNSCGCYSVSSVLPGCAERWNSGASFKCTVTHPESDTLTGTIAKVTVNTFPPQV
 HLLPPPSEELALNELVSLTCLVRAFNPKEVLVRWLHGNEELSPESYLVEPLKEPGE
 GATTYLVTSVLRVSAEIWKQGDQYSCMVGHEALPMNFTQKTIDRLSGKPTNVSVS
 VIMSEGD

25

A. mIgA WCH2 T4CH3

Nucleotide sequence:

Gttgtgatcacatctgttctccttactactcctcctccaccttctgccagcccagcctgtcactgcagcggccagctcttgagga
 cctgtcctgggttcagatgccagcatcacatgtactctgaatggcctgagagatcctgagggagctgtcttccctgggagccctc
 30 cactgggaaggatgcagtgacagaagaaagctgtgcagaattcctgcggctgctacagtgtgccagcgtcctgacctggtgtgtg
 agcgtggaacagtggcgcatcattcaagtgcacagttaccatcctgagctgtacaccttaactggcacaattgccaaagtcaca
 gtgaacaccttcccaccccaggtccacctgtaccgccgccgtcggaggagctggccctgaatgagctcgtgtccctgacatgcc
 tgggtgcgagcttcaaccctaaagaagtgtggtgcgatggctgcagtgaaatgaggagctgtccccagaaagctacctagtgttg
 agcccctaaaggagccaggcgaggagccaccacctacctggtgacaagcgtgttgcgtgtatcagctgaaatctggaacagg
 35 gtgaccagtactcctgcatgtgtgggccacgaggccttgcccatgaacttcaccacagaagaccatcgaccgtctgtcgggttaaacc
 caccaatgtcagcgtgtctgtgatcatgtcagaggagattgataatctagat

Amino acid sequence:

40 DHICSPPTTPPPPSCQPSLSLQRPALDLLLLGSDASITCTLNGLRDPEGAVFTWEPST
 GKDAVQKKAVQNSCGCYSVSSVLPGCAERWNSGASFKCTVTHPESDTLTGTIAKV
 TVNTFPPQVHLLPPPSEELALNELVSLTCLVRAFNPKEVLVRWLHGNEELSPESYL
 VFEPLKEPGE GATTYLVTSVLRVSAEIWKQGDQYSCMVGHEALPMNFTQKTIDRL
 SGKPTNVSVSVIMSEGD

45 20. K322S CH2 region

Nucleotide sequence:

cctgaactcctggggggaccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcac
 atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttaactggtacgtggacggcgtggagggtgcataatgccaa
 gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatg
 50 gcaaggagtacaagtgtcgtctccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaa

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCSVSNKALPAPIEKTISKAK

Nucleotide sequence:

cttgaactcctggggggaccgtcagttcttcttccccccaaaacccaaggacacctcatgatctccgggacccctgaggtcac
atgctggtggtggacgtgagccacgaagacctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatg
gcaaggagtacaagtgtctcgttccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccc
cgagaaccacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtcaaagg
cttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctgg
actccgacggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtg
atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFKNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCSVSNKALPAPIEKTISKAKG
QPREPVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
LDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTOKSLSLSPGK

Nucleotide sequence:

tgatcaggagcccaaatctctgacaaaactcacacatccccaccgltctcagcacctgaactctctggggggaccgtcagtcttccct
cttcccccaaaacccaaggacacctcatgatctcccggacccttgaggtcacatgcgtggtggtggacgtgagccacgaaga
ccctgaggtcaagtcaactggtagctggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaaca
gcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcctggtctccaaca
agccctcccagcccccctcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccat
cccgggatgagctgaccaagaaccaggtcagcctgacctgcttggtcaaaggcttctatccagcgacatcgccgtggagtggg
agagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagct
caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacga
gaagagcctctccctgtctccgggtaaatgatctaga

DQEPKSSDKTHTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCLV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTOKSLSLSPGK

Nucleotide sequence:

aagcttccgccatgggattttcaagtgagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
ccagctctccagcaatcctgtctgcatactccaggggagaaggtcacaaatgacttgaggccagctcaagtgttaagtacatgcact
ggtaccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctctgctcgttcagtg
gcagtggggtctgggacctctactctctcacaatcagcagagtggagggtgaagatgctgccacttattactgccagcagtgaggattt
taaccacccacgcttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggagggtg
ggagctctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtggaagatgtcctgcaaggcttctggc
tacacattaccagttacaatatgcactgggtaaacgagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcag

cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactgggtacttcgatgtctggggcac
 agggaccacgggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaact
 cctgggggggaccgtcagttcttcttcccccaaaaccaaggacacccctcatgatctcccggaccctgaggtcacatgcgtgg
 5 tgggtggacgtgagccacgaagaccctgaggtcaagttaactgggtacgtggacggcgtggaggtgcataatgccaagacaaagc
 cgcgaggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggag
 tacaagtgtcgggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaacca
 caggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccca
 gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacgg
 10 ctcttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgatgagg
 ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QKPGSSPKPWYAPS NLASGV PARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 15 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSSAPELLGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCSVSNKALPAPIE
 20 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSGDFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
 PGK

23. 2H7 scFv VHL11S hIgG1 (SSS-S)H K322LCH2 WCH3

Nucleotide sequence:

aagcttgcgccatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctct
 ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacatgacttcagggccagctcaagtgttaagtacatgcact
 30 ggtaccagcagaagccaggatcctccccaaacctggatttatgcccatccaacctggcttctggagtcctgtcgcgttcagtg
 gcagtgggtctgggaccttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggatt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggatctggaggaggtg
 ggagctctcaggttatctacagcagctggggctgagtcgggtgagcctggggcctcagtgaaatgtcctgcaaggcttctggc
 tacacatttaccagttacaatatgcactgggtaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 35 ggtgatacttctacaatcagaagttcaagggaagggcacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactgggtacttcgatgtctggggcac
 agggaccacgggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaact
 cctgggggggaccgtcagttcttcttcccccaaaaccaaggacacccctcatgatctcccggaccctgaggtcacatgcgtgg
 tgggtggacgtgagccacgaagaccctgaggtcaagttaactgggtacgtggacggcgtggaggtgcataatgccaagacaaagc
 cgcgaggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggag
 40 tacaagtgcctggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaacca
 caggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccca
 gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacgg
 ctcttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgatgagg
 45 ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QKPGSSPKPWYAPS NLASGV PARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 50 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK

THTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCLVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
 PGK

24. 2H7 scFv VHL11S hIgG1 (CSS-S)H K322SCH2 WCH3

Nucleotide sequence:

aagcttccgccatggatttcaagtgcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctct
 10 cccagctctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttcagggccagctcaagtgttaagttacatgact
 ggtaccagcagaagccaggatctcccccaaacctggatttatccccatccaacctggcttctggagtcctgctgcttcagtg
 gcagtggtctgggaccttactcttcacaatcagcagagtgaggctgaagatgctgccacttattactgccagcagtgaggttt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctctgggctgagtcggtgaggcctggggacctcagtgagatgctcctgcaaggcttctggc
 15 tacacattaccagttacaatatgactgggtaaagcagacacctagacaggcctggaatggattggagctattatccaggaaat
 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggtctatttctgtcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
 agggaccacggtcaccgtctcttctgatcaggagcccaaatctgtgacaaaactcacatccccaccgtcctcagcacctgaact
 cctgggggggaccgtcagcttctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtgg
 20 tgggtgagcgtgagccacgaagaccctgaggtcaagttaactgggtacgtggagcggcgtggaggtgcataatgccaaagacaaagc
 cgcgggaggagcagtaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactgggtgaatggcaaggag
 tacaagtgtcgggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaacca
 caggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgctcaaggtcttatccca
 gcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctccgtgctggactccgacgg
 25 ctctcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagtagg
 ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 30 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSCDK
 THTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 35 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCSVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
 PGK

25. P331S CH2

Nucleotide sequence:

cctgaactcctggggggaccgtcagcttctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcac
 atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaa
 gacaaagccgaggagcagtaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactgggtgaatg
 45 gcaaggagtacaagtgaaggtctccaacaaagccctcccagcctccatcgagaaaacaatctccaagccaaa

Amino acid sequence

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAK

26. P331S CH2 WCH3

Nucleotide sequence:

cctgaactcctgggggaccgtcagttcttcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcac
 atgctgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaactggtagctggacggcgtggaggtgcataatgcaa
 gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactgggtgaatg
 5 gcaaggagtacaagtgaaggtctccaacaaagccctcccagcctccatcgagaaaacaatctcaaagccaaagggcagccc
 cgagaaccacaggtgtacacctgccccatcccggtgagctgaccaagaaccaggtcagcctgacctgctgtcaaagg
 ctctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctgg
 actccgacggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtg
 atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKG
 QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
 15 LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

27. 2H7 scFv VH L11S (SSS-S)H P331S CH2 WCH3

Nucleotide sequence:

aagcttgccgcatggaatttcaagtgcagatttccagttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 20 cccagttccagcaatcctgtctgcattctccaggggagaaggtcacaatgactgcagggccagctcaagtgaattacatgcact
 ggtaccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtg
 gcagtggtgtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagttt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctcgggctgagtcggtgaggcctggggcctcagtgaaatgctctgcaaggcttctggc
 25 tacacattaccagttacaatatgcactgggtaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgctggggcac
 agggaccacggtcaccgtctctctgatcaggagcccaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaact
 cctgggggggaccgtcagttcttcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgg
 30 tgggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaagc
 cgcgaggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggtgaatggcaaggag
 tacaagtgaaggtctccaacaaagccctcccagcctccatcgagaaaacaatctcaaagccaaagggcagccccgagaacca
 caggtgtacacctgccccatcccggtgagctgaccaagaaccaggtcagcctgacctgctgtcaaaggcttctatccca
 gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacgg
 35 ctcttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgagg
 ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 40 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVYYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 45 THTSPSSAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 NYKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
 PGK

28. 2H7 scFv VH L11S (CSS-S)H P331S CH2 WCH3

Nucleotide sequence:

aagcttgcgccatggatttcaagtgcagatttcagcttctgtaatcagtgcttcagtcataattgccagaggacaaattgttctct
 cccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagttacatgcact
 5 ggtaccagcagaagccaggtatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
 gcagtggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggattt
 taaccacccacggtcgggtgctgggaccaagctggagctgaaagatggcggtggctcgggcgggtggtgatctggaggaggtg
 ggagctctcaggcttattctacagcagctcggggctgagtcgggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggc
 tacacattaccagttacaatatgcactgggtaagcagacacctagacagggcctggaatggattggagctatttatccaggaat
 ggtgatacttctacaatcagaagtcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcag
 10 cagcctgacatctgaagactctcggtctatttctgtgcaagagtgggtactatagtaacttactggtacttogatctctggggcac
 agggaccacgggtcaccgtctctctgatcaggagcccaatctgtgacaaaactcacacatccccaccgtcctcagcacctgaact
 cctggggggaccgtcagcttctctctcccccaaaaccaaggacacctcatgatctcccggaccctgaggtcacatgcgtgg
 tgggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc
 cgcgggaggagcagtagacaacagcacgtaccgtgtgtgagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
 15 tacaagtgaagggtctcaacaaagccctccagcctccatcgagaaaacaatctccaaagccaaagggcagccccgagaacca
 caggtgtacacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgacctggtcaaggcttctatccca
 gcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctggactccgacgg
 ctcttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgagg
 ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 25 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSA VYFCARVVYYSNSYWYFDVWGTGTTTVTVSSDQEPKSCDK
 THTSPSSAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPN
 30 NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
 PGK

29. T256N CH2 region

Nucleotide sequence:

35 Cctgaactcctggggggaccgtcagttcttcttcccccaaaaccaaggacacctcatgatctcccgaacctgaggtca
 catgcgtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaa
 agacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat
 ggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcgagaaaacaatctccaagccaaa

Amino acid sequence

40 PELLGGPSVFLFPPKPKDTLMISRNPEVTCVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

30. T256N CH2 WCH3

Nucleotide sequence:

45 cctgaactcctggggggaccgtcagttcttcttcccccaaaaccaaggacacctcatgatctcccgaacctgaggtcac
 atgcgtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaa
 gacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
 gcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcgagaaaacaatctccaagccaaaggcgagccc
 50 cgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgacctggtcaaagg
 ctctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctgg

actccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtg
atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

5 PELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK

10 31. 2H7 scFv VH L11S (SSS-S)H T256N CH2 WCH3

Nucleotide sequence:

aagcttgcgcgatgattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctct
cccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagttaagttacatgcact
15 ggtaccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtg
gcagtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggttt
taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtg
ggagctctcaggttatctacagcagcttggggctgagtcgggtgagcctggggcctcagtgaaagatgtcctgcaaggcttctggc
tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaat
20 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcgggtctattctgtgcaagagtgggtactatagtaacttactggtacttcgatgtctggggcac
agggaccacgggtcaccgtctctctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtcctcagcacctgaact
cctgggggggaccgtcagcttctcttcccccaaaaccaaggacacccctcatgatctcccgaacctgaggtcacatgcgtgg
tggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggagggtgcataatgccaagacaaagc
25 cgcgggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactgggtgaatggcaaggag
tacaagtgcaaggtctccaacaaagccctccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaacc
acaggtgtacaccctgcccccatccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaaggcttctatccc
agcgacatcgccgtggagtgggagagcaatgggcagccgggagaacaactacaagaccacgcctcccgtgtggactccgacg
gtccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgcagtag
30 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
35 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
THTSPSSAPELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
40 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSL
SPGK

45 32. 2H7 scFv VH L11S (CSS-S)H T256N CH2 WCH3

Nucleotide sequence:

aagcttgcgcgatgattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctct
cccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagttaagttacatgcact
50 ggtaccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtg
gcagtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggttt
taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtg

ggagctctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
 tacacatttaccagttacaatatgactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggctctatttctgtgcaagagtgggtgtactatagtaactcttactggtacttcgatgtctggggcac
 5 agggaccacggtcaccgtctcttctgatcaggagcccaaatcttgtgacaaaactcacacatccccaccgtcctcagcacctgaact
 cctgggggggaccgtcagtcttctcttcccccaaaaacccaaggacaccctcatgatctcccgaaccctgaggtcacatgcgtgg
 tggtagcgtgagccacgaagaccctgaggtcaagttcaactggtagcgtggacggcgtggaggtgcataatgccaaagacaagc
 cgcgaggaggagcagtacaacagcacgtaccgtgtggtagcgtctcaccgtcctgcaccaggactgggtgaatggcaaggag
 tacaagtgaaggctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaacc
 10 acaggtgtacaccctgccccatcccggtgatgactgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatccc
 agcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccggtgctggactccgacg
 gctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagag
 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

15 Amino acid sequence

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 20 TAYMQLSSLTSEDSAVYFCARVVYYNSYWYFDVWGTGTTVTVSSDQEPKSCDK
 THTSPSSAPELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSL
 25 SPGK:

33. RTPE/QNAK (255-258) CH2

Nucleotide sequence:

cctgaactcctggggggaccgtcagtcttctcttcccccaaaaacccaaggacaccctcatgatctcccagaacgtaagggtcac
 30 atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
 gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtagcgtcctcaccgtcctgcaccaggactggctgaatg
 gcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaa

Amino acid sequence

35 PELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

34. RTPE/QNAK (255-258)CH2 WCH3

Nucleotide sequence:

cctgaactcctggggggaccgtcagtcttctcttcccccaaaaacccaaggacaccctcatgatctcccagaacgtaagggtcac
 40 atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
 gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtagcgtcctcaccgtcctgcaccaggactggctgaatg
 gcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccc
 cgagaaccacaggtgtacaccctgccccatcccggtgatgactgaccaagaaccaggtcagcctgacctgcctggtcaaagg
 45 ctctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccggtgctgg
 actccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtg
 atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

50 PELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG

QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

35. 2H7 scFv VH L11S (SSS-S)H RTPE/QNAK (255-258)CH2 WCH3

Nucleotide sequence:

aagcttgccgccatggatttcagtgagatttcagcttccctgctaatactgcttcagtcataattgccagaggacaaattgttctct
 cccaggtctccagcaatcctgtctgactatccaggggagaaggtcacaaatgacttgcaggggccagctcaagtgttaagtacatgcact
 ggtaccagcagaagccaggatcctccccaaccctggatttatgccccatccaacctggcttctggagtccttgctcgttcagt
 gcagtgggtctgggacctcttactcttcacaatcagcagagtggaggtggaagatgctgccacttattactgccagcagtggaagtt
 taaccacccacggtcgggtgctgggaccaagctggagctgaaagatggcggtggctcgggcgggtggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggc
 tacacattaccagttacaatatgactgggtaaacgagacacctagacagggcctggaatggattggagctattatccaggaaat
 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggctctattctgtgcaagagtgggtgactatagtaactcttactggtaactcgatgctcggggcac
 agggaccacggtcaccgctcttctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtcctcagcacctgaact
 cctgggggggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctccagaacgctaaggtcacatgcgtgg
 tgggtggacgtgagccacgaagaccctgaggtcaagttcaactgggtacgtggacggcgtggaggtgcataatgccaagacaaagc
 cgcgaggaggagcagtacaacagcacgtaccgtgtgggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggag
 tacaagtgaaggtctccaacaaaggccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaacc
 acaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcttggtcaaaggcttctatccc
 agcgacatcgccgtggagtgaggagagcaatgggcagccggagacaactacaagaccacgcctcccggtgctggactccgacg
 gctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgatgag
 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVILARGQIVLSQSPAILSASPGKEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSSAPELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
 SPGK

36. 2H7 scFv VH L11S (CSS-S)H RTPE/QNAK (255-258)CH2 WCH3

Nucleotide sequence:

aagcttgccgccaatggattttcaagtgacagatttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
cccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttgcaggggccagctcaagtgttaagtacatgcact
ggtaccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgtctgccttcagt
gcagtggggtctgggacctcttactcttcacaatcagcagagtgagggtgaagatgtgccacttattactgccagcagtggaagttt
taaccacccacggttcggtgtctgggaccaagctggagctgaagatggcgggtggctcggggcgggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagctctggggctgagtcgggtgaggcctggggccctcagtgaaatgtcctgcaaggcttctggc
tacacattaccagttacaatatgcactgggtaaaagcagacacctagacagggccctggaatggattggagctatttatccaggaaat
ggtgatacttctacaatcagaagttcaagggccaaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcgggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtcttctgtatcaggagcccaaatcttgtacaaaactcacatccccaccgtcctcagcacctgaact
cctgggggggaccgtcagcttcttcttccccccaaaacccaaggacaccctcatgatctcccagaacgctaaggtcacatgcgtgg
tggtggacgtgagccacgaagaccctgaggtcaagttcaactgggtacgtggacggcgtggaggtgcataatgccaagacaaagc

cgcgaggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
 tacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaacc
 acaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggtcttatccc
 agcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
 5 gctccttctcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctctcatgctccgtgatgcatgag
 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 10 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSCDK
 15 THTSPSSAPPELLGGPSVFLFPPKPKDTLMISQNAKVTCTVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSL
 SPGK

20 36. K290Q CH2 region

Nucleotide sequence:

cctgaactcctggggggaccgtcagttcttcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcac
 atgcgtggtgggtggacgtgagccacgaagaccctgaggtcaagttaactgtacgtggacggcgtggaggtgcataatgcaa
 25 gacacagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
 gcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaa

Amino acid sequence:

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 30 AKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

37. K290Q CH2 WCH3

Nucleotide sequence:

Cctgaactcctggggggaccgtcagttcttcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtca
 catgcgtggtgggtggacgtgagccacgaagaccctgaggtcaagttaactgtacgtggacggcgtggaggtgcataatgcca
 35 agacacagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat
 ggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagcc
 ccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaag
 gcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctg
 40 gactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgt
 gatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 45 AKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
 QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
 LDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK

38. 2H7 scFv VH L11S (SSS-S)H K290Q CH2 WCH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtgagatttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctct
 50 cccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgagggccagctcaagtgaagtacatgcact

ggtagcagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagt
 gcagtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggatt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtgctcggcggtggtggatctggaggaggtg
 5 ggagctctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagtgtcctgcaaggcttctggc
 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaacttctactgtacttcgatgtctggggcac
 agggaccacgggtaccgtctcttctgatcaggagcccaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaact
 cctgggggggaccgtcagcttcttcttcccccaaaaccaaggacacctcatgatctccggacccctgaggtcacatgctgtg
 10 tgggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacacagc
 cgcgggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactgggtgaatggcaaggag
 tacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaacc
 acaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgctcaaaggcttctatccc
 agcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
 15 gctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 20 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 25 DGVEVHNAKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
 PGK

39. 2H7 scfv VH L11S (CSS-S)H K290Q CH2 WCH3

Nucleotide sequence:

aagcttgcgccatgattttcaagtgcagatttcagcttctgctaactcagtgcttcagtcataattgccagaggacaaattgttctct
 cccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgaattacatgcact
 35 ggtaccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagt
 gcagtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggatt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtgctcggcggtggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagtgtcctgcaaggcttctggc
 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 40 cagcctgacatctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaacttctactgtacttcgatgtctggggcac
 agggaccacgggtaccgtctcttctgatcaggagcccaatcttgtgacaaaactcacacatccccaccgtcctcagcacctgaact
 cctgggggggaccgtcagcttcttcttcccccaaaaccaaggacacctcatgatctccggacccctgaggtcacatgctgtg
 tgggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacacagc
 cgcgggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactgggtgaatggcaaggag
 45 tacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaacc
 acaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgctcaaaggcttctatccc
 agcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
 gctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
 50 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 5 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTTVTVSSDQEPKSCDK
 THTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 10 NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLS
 PGK

40. A339PCH2

Nucleotide sequence:

cctgaactcctggggggaccgtcagtccttcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcac
 15 atgcgtggtggtagcgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaa
 gacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
 gcaaggagtacaagtgaaggtctccaacaaagccctccagcccccatcgagaaaacaatctccaaacccaaa

Amino acid sequence:

20 PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKPK

41. A339P CH2 WCH3

Nucleotide sequence:

cctgaactcctggggggaccgtcagtccttcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcac
 atgcgtggtggtagcgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaa
 gacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
 gcaaggagtacaagtgaaggtctccaacaaagccctccagcccccatcgagaaaacaatctccaaacccaaaggcagccc
 30 cgagaaccacaggtgtacaccctgcccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaagg
 cttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccagcctcccgtgctgg
 actccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcgtg
 atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

35 PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKPKG
 QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
 40 LDDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK

42. 2H7 scFv VHL11S (SSS-S)H A339P CH2 WCH3

Nucleotide sequence:

aagcttgccgccatggatttcaagtgcagatttctcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctt
 cccagctctccagcaatctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcact
 45 ggtaccagcagaagccaggtaccccccacccctggattatgccccatccaacctggcttctggagtcctctgctcgttcagtg
 gcagtggtgtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggatt
 taaccacccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggtcgggaggtggtgagctgaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagtcgggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggc
 tacacattaccagttacaatatgactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaat
 50 ggtgatacttctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaactcttactgggtacttcgatgtctggggcac

agggaccacgggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatcccaccgtctcagcacctgaact
 cctggggggaccgtcagctcttcttcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtgg
 tggtagacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc
 cgcgaggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
 5 tacaagtgaaggtctccaacaaagccctcccagcccccacgcagaaaacaatctcaaaccctaaagggcagccccgagaacc
 acaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggctcaaaggcttctatccc
 agcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
 gctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
 gctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaatgatctaga

10

Amino acid sequence:

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 15 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYNSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKPKGQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 20 NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLS
 PGK

43. 2H7 scFv VHL11S (CSS-S)H A339P CH2 WCH3

25 Nucleotide sequence:

aagcttccgccatggatttcaagtgcagatttcagcttctgctaactcagtgcttcagtcataattgccagaggacaaattgttctct
 cccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcact
 ggtaccagcagaagccaggatcctccccaaacctggatttatgcccacccaacctggcttctggagtcctgctcgttcagtg
 gcagtggtctgggacctcttactcttcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggatt
 30 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtgagctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagtcgggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggc
 tacacattaccagttacaatatgactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
 ggtgatacttctacaatcagaagttcaagggcaaggccacactgactgtagacaaaatctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggcttattctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcac
 35 agggaccacgggtcaccgtcttctgatcaggagcccaaatcttctgacaaaactcacacatcccaccgtcctcagcacctgaact
 cctggggggaccgtcagcttcttcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtgg
 tggtagacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc
 cgcgaggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
 tacaagtgaaggtctccaacaaagccctcccagcccccacgcagaaaacaatctcaaaccctaaagggcagccccgagaacc
 40 acaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggctcaaaggcttctatccc
 agcgacatcgccgtggagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
 gctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
 gctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaatgatctaga

45 Amino acid sequence:

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 50 TAYMQLSSLTSEDSAVYFCARVVYYNSNSYWFYFDVWGTGTTVTVSSDQEPKSCDK
 THTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV

DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKPKGQPPEPVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPN
NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
PGK

5

44. G28-1VH

Nucleotide sequence:

gcggtccagctgcagcagctctggacctgagctggaaaagcctggcgctttagtgaagatttctgcaaggcttctggttactcattc
actggctacaatatgaactgggtgaagcagaataatgaaagagccttagtggattggaaatattgatccttattatggtggtacta
cctacaaccggaagtcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgac
atctgaggactctgcagcttattactgtgaagatcggtcgccctatggactactgggtgcaaggaacctcagtcaccgtctcttct
gatcag

15 Amino acid sequence:

AVQLQQSGPELEKPGASVKISCKASGYSTGYNMNWVKQNNGKSLEWIGNIDPY
YGGTTYNRKFKGKATLTVDKSSSTAYMQLKSLTSEDSAVYYCARSVGPMDYWG
QGTSVTVSSDQ

45. G28-1VL

Nucleotide sequence:

aagcttgcggccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatgtttacagttatttggcttggt
atcagcagaacagggaaaatctcctcagctcctggtctctttgcaaaaaccttagcagaaggtgtgccatcaagggtcagtggtgca
gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatcattccgataat
cctgtggacgttcggtggaggcaccgaactggagatcaaagggtggcggtggctcggcggtggtgggtcgggtggcgggcggt
cgtca

30 Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQKGKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSS

46. G28-1 scFv

Nucleotide sequence:

aagcttgcggccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatgtttacagttatttggcttggt
atcagcagaacagggaaaatctcctcagctcctggtctctttgcaaaaaccttagcagaaggtgtgccatcaagggtcagtggtgca
gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatcattccgataat
cctgtggacgttcggtggaggcaccgaactggagatcaaagggtggcggtggctcggcggtggtgggtcgggtggcgggcggt
cgtcagcggtccagctgcagcagcttgacctgagctggaaaagcctggcgctttagtgaagatttctgcaaggcttctggttact
cattactggctacaatatgaactgggtgaagcagaataatgaaagagccttagtggattggaaatattgatccttattatggtggt
actacctacaaccggaagtcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtct
gacatctgaggactctgcagcttattactgtgaagatcggtcgccctatggactactgggtgcaaggaacctcagtcaccgtctc
ttctgatcag

Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQKGKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPELEKPGASVKISCKA

SGYSFTGYNMNWVKQNNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
 AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQ

5 **47. G28-1 VHL11S**

Nucleotide sequence:

gcggtccagctgcagcagctctggacctgagtcggaaaagcctggcgcttcagtgaaagattcctgcaaggcttctggttactcattc
 actggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtgattggaaatattgatccttattatggtggtacta
 cctacaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctgac
 10 atctgaggactctgcagctctattactgtgcaagatcggtcggccctatggactactggggtcaaggaacctcagtcaccgtctctct
 gatcag

Amino acid sequence:

15 AVQLQQSGPESEKPGASVKISCKASGYSTGYNMNWVKQNNNGKSLEWIGNIDPYY
 GGTTYNRKFKGKATLTVDKSSSTAYMQLKSLTSEDSAVYYCARSVGPMDYWGQ
 GTSVTVSSDQ

20 **48. G28-1 VHL11S scFv**

Nucleotide sequence:

aagcttccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
 agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatgtttacagttatttggttggt
 atcagcagaacagggaaaatctcctcagctcctggtctcttttgcacaaaccttagcagaaggtgtgccatcaaggttcagtggtgca
 25 gtggatcaggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtcaacatcattccgataat
 ccgtggacgttcggtggaggcaccgaactggagatcaaggtggcggtggctcgggcggtggtgggtcgggtggcggcggtat
 cgtcagcgggtccagctgcagcagcttgacactgagtcggaaaagcctggcgcttcagtgaaagattcctgcaaggcttctggttact
 cattcactggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtgattggaaatattgatccttattatggtggt
 actacctacaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctc
 30 gacatctgaggactctgcagctctattactgtgcaagatcggtcggccctatggactactggggtcaaggaacctcagtcaccgtctc
 ttctgatcag

Amino acid sequence:

35 MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLA
 YQKQKQKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLPEDSGSYFCQHHS
 DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
 SGYSFTGYNMNWVKQNNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
 AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQ

40 **49. G28-1 scFv (SSS-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
 agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatgtttacagttatttggttggt
 atcagcagaacagggaaaatctcctcagctcctggtctcttttgcacaaaccttagcagaaggtgtgccatcaaggttcagtggtgca
 45 gtggatcaggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtcaacatcattccgataat
 ccgtggacgttcggtggaggcaccgaactggagatcaaggtggcggtggctcgggcggtggtgggtcgggtggcggcggtat
 cgtcagcgggtccagctgcagcagcttgacactgagtcggaaaagcctggcgcttcagtgaaagattcctgcaaggcttctggttact
 cattcactggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtgattggaaatattgatccttattatggtggt
 actacctacaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctc
 50 gacatctgaggactctgcagctctattactgtgcaagatcggtcggccctatggactactggggtcaaggaacctcagtcaccgtctc
 ttctgatcatgatcaggagcccaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggaccgtc

agttcttcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagcc
 acgaagacctgaggtcaagttcaactggtagctggacggcgtggaggtgcataatgccaagacaaagccgaggagga
 gtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaagggtc
 tccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacct
 5 gccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcgacatcgccgtg
 gagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctac
 agcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccact
 acacgcagaagagcctctccctgtctccgggtaaatgatctaga

10 Amino acid sequence:

MVSTAQFLGLLLWL TGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
 YQQKQKGKSPQLLV SFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
 DNPWTFGGGTELEIKGGGSGGGGSGGGGSSAVQLQQSGPELEKPGASVKISCKA
 SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
 15 AYMQLKSLTSEDSAVYYCARSVGPM DYWGQGTSTVTVSSDHDQEPKSSDKTHTSP
 PSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE
 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
 AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

20

50. G28-1 scFv IgAW H IgG1WCH2 WCH3

Nucleotide sequence:

aagcttccgccatggtatccacagctcagttccttgggttgctgctgctggtggttacaggtggcagatgtgacatccagatgactc
 agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatgtttacagttatttggttggt
 25 atcagcagaacagggaaaatctctcagctcctggtctcttttgcanaaaccttagcagaaggtgtccatcaagggtcagtgga
 gtggatcaggcacacagtttctctgaagatcagcagcctgcagcctgaagattctggaagttattctgtcaacatcattccgataat
 ccgtggacgttcggtggaggcaccgaactggagatcaaagggtggcgggtggctcgggcgggtggtgggtcgggtggcggcggt
 cgtcagcgggtccagctgcagcagcttgacctgagctggaaaagcctggcgcttcagtgaaattctcgaaggctctggttact
 cattcactggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggaattggaatattgatccttattatggtggt
 30 actacctacaaccggaagtcaagggaaggccacattgactgtagacaaatctccagcacagcctacatgcagctcaagagct
 gacatctgaggactctgcagcttattactgtgcaagatcggtcggccctatggactactgggtcaaggaacctcagtcaccgtctc
 ttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgcgcacctgaactcctgg
 ggggacctgagcttctcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtg
 gacgtgagccacgaagacctgaggtcaagttcaactggtagctggacggcgtggaggtgcataatgccaagacaaagccgcg
 35 ggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa
 gtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacagg
 gtacacctgccccatcccggtgatgctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcga
 catcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcct
 tcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctg
 40 cacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MVSTAQFLGLLLWL TGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
 YQQKQKGKSPQLLV SFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
 45 DNPWTFGGGTELEIKGGGSGGGGSGGGGSSAVQLQQSGPELEKPGASVKISCKA
 SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
 AYMQLKSLTSEDSAVYYCARSVGPM DYWGQGTSTVTVSSDQVPSTPPTPSPSTPPT
 PSPSCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDG
 VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 50 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY

KTTTPVLDSDFSFLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPG
K

51. **G28-1 scFv VHL11S (SSS-S)H WCH2 WCH3**

5 Nucleotide sequence:

aagcttgcgcgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
agtcctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaataatgtttacagttatttggcttggt
atcagcagaaacagggaaaatctctcagctccttggtctcttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtgga
gtggatcaggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagttatttctgcaacatcattccgataat
10 ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcggcggtggtgggtcgggtggcgcggtat
cgtcagcggtccagctgcagcagcttgacactgagtcggaaaagcctggcgcttcagtgaaagtttctgcaaggcttctggttact
cattcactggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtgattggaaatattgatccttattatggtggt
actacctacaaccggaagttcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctc
gacatctgaggactctgcagcttattactgtgcaagatcggtcggccctatggactactgggtgcaaggaaacctcagtcaccgtctc
15 ttctgatcaggagcccaaatcttctgacaaaactcacacatcccaccgtcctcagcacctgaactcctggggggaccgtcagctctt
cctcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacgaa
gacctgaggtcaagttcaactggtacgtggacggcggtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaa
cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccagactggctgaatggcaaggagtacaagtgaaggtctccaac
aaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccc
20 atccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaaggcttctatccagcgacatcgccgtggagtggt
gagagcaatgggcagccggagaacaactacaagaccagcctccgtgctggactccgacggctccttctctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctcctgtctccgggtaaatgatctaga

25 Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQKGKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
30 AYMQLKSLTSEDSAVYYCARSVGPMDYWGQTSVTVSSDHDQEPKSSDKTHTSP
PSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDFSFLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK
35

52. **G28-1 scFv VHL11S (CSS-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttgcgcgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
agtcctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaataatgtttacagttatttggcttggt
40 atcagcagaaacagggaaaatctctcagctccttggtctcttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtgga
gtggatcaggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagttatttctgcaacatcattccgataat
ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcggcggtggtgggtcgggtggcgcggtat
cgtcagcggtccagctgcagcagcttgacactgagtcggaaaagcctggcgcttcagtgaaagtttctgcaaggcttctggttact
cattcactggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtgattggaaatattgatccttattatggtggt
45 actacctacaaccggaagttcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctc
gacatctgaggactctgcagcttattactgtgcaagatcggtcggccctatggactactgggtgcaaggaaacctcagtcaccgtctc
ttctgatcaggagcccaaatcttctgacaaaactcacacatcccaccgtcctcagcacctgaactcctggggggaccgtcagctctt
cctcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacgaa
gacctgaggtcaagttcaactggtacgtggacggcggtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaa
50 cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccagactggctgaatggcaaggagtacaagtgaaggtctccaac
aaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccc

atccccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcgacatcgccgtggagtgg
gagagcaatgggcagccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

5

Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
10 SGYSFTGYNMNWKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSTVTVSSDQEPKSCDKTHTSPSS
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
15 VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

53. G28-1 scFv VH L11S (CSC-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgcgcccatggtatccacagctcagttccttgggtgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
20 agtctccagcctccctatctgcatctgtggagagactgtccatcacatgtcgaacaagtgaatgtttacagttatttggttggt
atcagcagaaacagggaaaatctcctcagctcctggtctcttttgcacaaaccttagcagaaggtgtgccatcaaggtcagtgga
gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagttatttctgcaacatcattccgataat
ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcggcggtggtgggtcgggtggcgcggtg
cgtcagcgggtccagctgcagcagctgtggacctgagtcggaaaagcctggcgctcagtggaagatttctgcaaggctctggttact
25 cattactggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtggaattggaatattgatccttattatggtggt
actacctacaaccggaaggtcaagggaaggccacatgactgtagacaaatcctccagcacagcctacatgcagctcaagagctc
gacatctgaggactctgcagctctattactgtgcaagatcggtcggccctatggactactggggtcaaggaaacctcagtcaccgtctc
ttctgatcaggagcccaaatcttgtgacaaaactcacacatctccaccgtgctcagcacctgaactcctgggtggaccgtcagttctc
ctctccccccaaaacccaaggacacctcatgatctcccggaccttgaggtcacatgcgtgggtgggtgacgtgagccacgaag
30 acctgagggtcaagttcaactggtacgtggacggcggtggaggtgcataatgccaagacaaagccgaggagagcagtagaac
agcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaaca
aagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccca
tccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccaagcgacatcgccgtggagtgg
gagagcaatgggcagccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttctctctacagcaag
35 ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
40 YQQKQGKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSTVTVSSDQEPKSCDKTHTSPPC
SAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
45 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
PVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

50

54. G28-1 scFv VH L11S (SSC-P)H WCH2 WCH3

Nucleotide sequence:

aagcttccgccaatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
 agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatgtttacagttatttggcttgg
 atcagcagaaacagggaaaatctctcagctcctgtctctttgcaaaaacctagcagaaggtgtgccatcaagggtcagtgga
 gtggtacaggcacacagtttctctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtcaacatcattccgataat
 5 ccgtggacgttcggtggaggcaccgaactggagatcaaagggtggcgggtggtcgggagggtggtgggtcgggtggcgccggat
 cgtcagcgggtccagctgcagcagcttgacctgagtcggaaaagcctggcgcttcagtgaagattcctgcaaggcttctggttact
 cattcactggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggattggaatatgtatcttattatggtggt
 actacctacaaccggaagtcaagggaaggccacattgactgtagacaaatctccagcacagcctacatgcagctcaagagtct
 gacatctgaggactctgcagcttattactgtgcaagatcggtcggccctatggactactgggtcaaggaaacctcagtcaccgtctc
 10 tctgtatcaggagcccaaatctctgacaaaactcacacatccccaccgtgccagcacctgaactcctggggggaccgtcagctctt
 cctcttcccccaaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtggtggtggacgtgagccacgaa
 gacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaaagccgcgggaggagcagtacaa
 cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaac
 aaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccc
 15 atcccggtatgagctgaccaagaaccaggtcagcctgacctgctggtcaaggcttctatccagcgacatcgccgtggagtgg
 gagagcaatgggcagccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttctctctacagcaag
 ctaccgtggacaagagcaggtggcagcaggggaacgtctctcatgctcgtgatgcatgaggctctgcacaaccactacacgc
 agaagagcctctcctgtctccgggtaaatgatctaga

Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLA
 YQKQKQKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLPEDSGSYFCQHHS
 DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKJSCA
 SGYSFTGYNMNWKQNNKSL EWIGNIDPYGGTTYNRKFKGKATLTVDKSSST
 25 AYMQLKSLTSEDSAVYYCARSVGPM DYWGQTSVTVSSDQEPKSSDKTHTSPPCP
 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
 GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
 VLDS DGSFFLYSKLTVDKSRWQQGNV FCSVMHEALHNHYTQKSLSLSPGK

II. 54. HCTLA4 HIGG1 (SSS-S)H P238SCH2 WCH3

Nucleotide sequence:

atggcttgcttgattcagcggcacaaggctcagctgaacctggctgccaggacctggccctgcactctcctgtttttcttctcttc
 atccctgtcttctgcaaagcaatgcacgtggcccagcctgctgtggtactggccagcagccaggcatcgccagcttctgtgtga
 35 gtatgcactccaggcaaaagccactgaggtccgggtgacagtcttcggcaggctgacagccaggtgactgaagtctgtcgccg
 aacctacatgacggggaatgagttgaccttcttagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactat
 ccaaggactgagggccatggacacgggactctacatctgcaagggtggagctcatgtaccaccgccatactacctgggcatagg
 caacggaaccagatttatgtaattgatccagaacctgcccagattctgatcaacccaaatcttctgacaaaactcacacatcccca
 ccgtcctcagcacctgaactcctggggggatcgtcagttcttcttcccccaaaaacccaaggacaccctcatgatctcccgac
 40 cctgaggtcacatgcgtggtggtgacgtgagccacgaagaccctgaggtcaagtcaactgggtacgtggacggcgtggaggt
 gcataatgccaagacaaaagccgcgggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccagg
 actggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagcca
 aagggcagccccgagaaccacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagctgacctgc
 ctggtcaaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcc
 45 tccgtgctgactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct
 catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaatga

Amino acid sequence:

MACLGFRHKAQLNLAARTWPCTLLFLLFIPVFCKAMHVAQPAVVLASSRGIAS
 50 FVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMTGNELTFLDDSICTGTSSGN

QVNLTIQGLRAMDTGLYICKVELMYPPPYLIGINGTQIYVIDPEPCPDSDQPKSSD
 KTHTSPSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 5 NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSL
 SPGK

55. Fc2-2 VL

Nucleotide sequence:

10 gttgtaagcttcccgcattgattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatg
 tcacagtctccatctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtcagtcagagcctttatataatcacaat
 caaaagaactacttggcctggtaccagcagataaccagggcagtcctctaaactgctgatttactgggcatccactagggaatctgg
 ggtccctgatcgttcacaggcagtggtatctgggacagattcactctcaccatcagcagagtgaaggctgaagacctggcagttta
 ttactgtcagcaatattatacctatcctccacgttcggaggtggcaccaagctggaaataaaagggtggcgggtggtcgggcgggtg
 15 gtgggtcgggtggcggcgggagctcg

Amino acid sequence:

MDSQAQVLMMLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
 QKNYLAWYQQIPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRKAEDLA
 20 VYYCQQYYTYPTFGGGTKLEIKGGGSGGGGSGGGGSS

56. FC2-2VH

Nucleotide sequence:

25 Gggagctcgcaggtgcagttgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtctca
 ggttctcaftaaccgtctatggtttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatggggtgat
 ggaagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaagtttcttaaaaalggac
 agtctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactgggggtcaagga
 acctcagtcaccgtctcctctgatcag

30 Amino acid sequence:

GSSQVQLKESGPGLVAPSQSLSTCTVSGFSLTVYGVNWVRQPPGKGLDWLGMTW
 GDGSTDYNSALKSRLSISKDNSKSQVFLKMDSLQTDRTARYYCARDHYGTHYAM
 DYWGQGTSTVTVSSDQ

57. FC2-2scFv

Nucleotide sequence:

35 gttgtaagcttcccgcattgattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatg
 tcacagtctccatctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtcagtcagagcctttatataatcacaat
 caaaagaactacttggcctggtaccagcagataaccagggcagtcctctaaactgctgatttactgggcatccactagggaatctgg
 40 ggtccctgatcgttcacaggcagtggtatctgggacagattcactctcaccatcagcagagtgaaggctgaagacctggcagttta
 ttactgtcagcaatattatacctatcctccacgttcggaggtggcaccaagctggaaataaaagggtggcgggtggtcgggcgggtg
 gtgggtcgggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggcctggtggcgccctcacagagcctgtcc
 atcacatgcaccgtctcaggttctcattaaccgtctatggtttaactgggttcgccagcctccaggaaagggtctggactggctgg
 gaatgatatggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaa
 45 gttttcttaaaaatggacagctctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatgg
 actactgggggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence:

50 MDSQAQVLMMLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
 QKNYLAWYQQIPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRKAEDLA
 VYYCQQYYTYPTFGGGTKLEIKGGGSGGGGSGGGGSSQVQLKESGPGLVAPSQ

SLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIWGDGSTDYNSALKSRLSISK
DNSKSQVFLKMDSLQTD TARYYCARDHYGTHYAMDYWGQGTSVTVSSDQ

58. FC2-2 VHL11S

5 Nucleotide sequence:

gggagctctcaggtgcagttgaaggagtcaggacctggctcgggtggcgccctcacagagcctgtccatcacatgcaccgtctcag
ggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatggggtgatg
gaagcacagactataattcagctctcaaaccagactgagcatcagtaaggacaactccaagagccaagttttctaaaaatggaca
gtctacaaactgatgacacagccagggtactactgtgccagagatcactatggtaccactatgctatggactactggggtcaaggaa
10 cctcagtcaccgtctcctctgatcag

Amino acid sequence:

(GSS)QVQLKESGPGSVAPSQSLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMI
WGDGSTDYNSALKSRLSISKDNSKSQVFLKMDSLQTD TARYYCARDHYGTHYA
15 MDYWGQGTSVTVSSDQ

59. FC2-2 VH L11S scFv

Nucleotide sequence:

ggtgttaagcttggcgcctggttcacaggccaggttcttatgttactgctgctatgggtatctgttacctgtggggacattgtgatg
20 tcacagtctccatctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtcagtcagagcctttatataatcacaat
caaaagaactacttggcctggtaccagcagataccaggggcagctcctaaactgctgattactgggcatccactagggaaatctgg
ggtccctgatcgttcacaggcagtgatctgggacagattcactctcaccatcagcagagtgaaggctgaagacctggcagttta
ttactgtcagcaatattatcctatctcccacgttcggaggtggcaccaagctggaaataaaagggtggcgggtggctcgggcgggtg
gtgggtcgggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggctcgggtggcgccctcacagagcctgtcc
25 atcacatgcaccgtctcagggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgg
gaatgatatggggtgatggaagcacagactataattcagctctcaaaccagactgagcatcagtaaggacaacccaagagccaa
gttttctaaaaatggacagctctacaaactgatgacacagccagggtactactgtgccagagatcactatggtaccactatgctatgg
actactggggtcaaggaaacctcagtcaccgtctcctctgatcag

30 Amino acid sequence:

MDSQAQVLM LLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
QKNYLAWYQQIPGQSPKLLIYWASTRESGV PDRFTGSGSGTDFTLTISR VKAEDLA
VYYCQQYYTYPPTFGGGTKLEIKGGGSGGGGSGGGGSSQVQLKESGPGSVAPSQ
SLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIWGDGSTDYNSALKSRLSISK
35 DNSKSQVFLKMDSLQTD TARYYCARDHYGTHYAMDYWGQGTSVTVSSDQ

60. FC2-2 (SSS-S)H WCH2 WCH3

Nucleotide sequence:

ggtgttaagcttggcgcctggttcacaggccaggttcttatgttactgctgctatgggtatctgttacctgtggggacattgtgatg
40 tcacagtctccatctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtcagtcagagcctttatataatcacaat
caaaagaactacttggcctggtaccagcagataccaggggcagctcctaaactgctgattactgggcatccactagggaaatctgg
ggtccctgatcgttcacaggcagtgatctgggacagattcactctcaccatcagcagagtgaaggctgaagacctggcagttta
ttactgtcagcaatattatcctatctcccacgttcggaggtggcaccaagctggaaataaaagggtggcgggtggctcgggcgggtg
gtgggtcgggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggcctggtggcgccctcacagagcctgtcc
45 atcacatgcaccgtctcagggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgg
gaatgatatggggtgatggaagcacagactataattcagctctcaaaccagactgagcatcagtaaggacaacccaagagccaa
gttttctaaaaatggacagctctacaaactgatgacacagccagggtactactgtgccagagatcactatggtaccactatgctatgg
actactggggtcaaggaaacctcagtcaccgtctcctctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtcct
cagcacctgaactcctgggtggaccgtcagcttctcttcccccaaaacccaaggacacctcatgatctccggacccttgag
50 gtcacatgcgtgggtggacgtgagccacgaagacctgaggtcaagttcaactgtacgtggacggcgtggaggtgcataat
gccaagacaaagccgcgggaggagcagtaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggct

gaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggc
agccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctatccaagcgacatcgccgtggagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctcccg
ctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
5 cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MDSQAQVLMMLLLWVSGTCGDIVMSQSPSSLA VSVGEKVSMSCCKSSQSLLYNHN
QKNYLA WYQQIPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISR VKAEDLA
10 VYYCQQYYTYPTFGGGTKLEIKGGGSGGGGSGGGGSSQVQLKESGPGLVAPSQ
SLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIWGDGSTDYNSALKSRLSISK
DNSKSQVFLKMDSLQTD TARYYCARDHYGTHYAMDYWGQGTSTVTVSSDQEPK
SSDKTHTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
15 LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQ
KSLSLSPGK

61. FC2-2 VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

gttgtaagcttccgccatggattcacaggcccaggttctatgttactgctgctatgggtatctggtacctgtggggacattgtgatg
tcacagtctccatctccctagctgtgtcagttggagagaaggttctatgagctgcaagtcagtcagagccctttatataatcacaat
caaaagaactacttggcctgtaccagcagataccaggggcagctcctctaaactgctgattactgggcatccactaggggaatctgg
25 ggtccctgatcgcttcacaggcagtgatctgggacagattcactctcaccatcagcagagtgaaggctgaagacctggcagttta
ttactgtcagcaatattatactatctccacgttcggaggtggcaccaagctggaataaaaagggtggcgggtggctcgggcgggtg
gtgggtcgggtggcggcgaggctctcaggtgcagtlgaaggagtcaggacctggctcgggtggcgccctcacagagcctgtcc
atcatatgcaccgtctcagggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgg
gaatgatatggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaa
gttttcttaaaaatggacagctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatgg
30 actactggggtcaaggaaacctcagtcaccgtctcctctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtct
cagcacctgaactcctgggtggaccgtcagcttctctctccccccaaaacccaaggacacctcatgatctcccggaacctgag
gtcacatgcgtgggtgggtggacgtgagccacgaagacctgaggtcaagttaactggtacgtggacggcgtggaggtgcataat
gccaagacaaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct
gaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggc
35 agccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctatccaagcgacatcgccgtggagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctcccg
ctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MDSQAQVLMMLLLWVSGTCGDIVMSQSPSSLA VSVGEKVSMSCCKSSQSLLYNHN
QKNYLA WYQQIPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISR VKAEDLA
45 VYYCQQYYTYPTFGGGTKLEIKGGGSGGGGSGGGGSSQVQLKESGPGSVAPSQ
SLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIWGDGSTDYNSALKSRLSISK
DNSKSQVFLKMDSLQTD TARYYCARDHYGTHYAMDYWGQGTSTVTVSSDQEPK
SSDKTHTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQ
50 KSLSLSPGK

62. UCHL-1 VH

Nucleotide sequence:

atgggcaggcttacttcttctgctactgattgttctgcatatgtcctctccagattactctgaaagagtctggccctgggatctt
 gcagccctcccagaccctcagctgacttgttcttctggttttctactgaccacttatggtataggagtaggttgattctgcagcct
 5 ccagggaagggtctggagtggctgacacacatttgggtgaatgataataagtactataacacagccctgaggagccggctcaca
 tctccaaggattctccaacaaccaagtactcctcaagatcgccaatgtggacactgcagataccgccacatactactgtctctacg
 gctacacttactggggccaagggactctggctactgtctctgca

Amino acid sequence:

10 MGRLTSSFLLLIVPAYVLSQITLKESGPGILQPSQTLSTCSFSGFSLTTYGIGVGWIR
 QPPGKGLEWLTHIWWNDNKYYNTALRSRLTISKDSSNNQVLLKIANVDTADTAT
 YYCLYGYTYWGQGLVTVSA

63. UCHL-1 VL

Nucleotide sequence:

atgaagttgcctgttaggctgttgggtgctgatgttctggattcctgctccatcagtgatgttgtgatgacccaaactccactctccctgc
 ctgtcagctctggagatcaggcctccatctcttgcagatctagtcagagccttcttacagtaatgaaacacctatttaccattggtacct
 gcagaagccaggccagctctcaaaaactcctgatctacaaactttcaaccgattttctgggggtcccagacaggttcagtggtcagtg
 atcaggggacagatttcacactcaagatcagcagagtgagggtgaggatctgggagttatttctgctctcaagtacacatgttccg
 20 tggacgttcggtggaggcaccagctggaaatcaaa

Amino acid sequence:

25 MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
 HWYLQKPGQSPKLLIYKLSNRFSGVSPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
 QSTHVPWTFGGGTKLEIK

64. UCHL-1 scFv

Nucleotide sequence:

gttgtaagcttgcggccatgaagttgcctgttaggctgttgggtgctgatgttctggattcctgctccatcagtgatgttgtgatgaccu
 30 aaactccactctccctgctgtcagctctggagatcaggcctccatctcttgcagatctagtcagagccttcttacagtaatgaaac
 acctatttaccattggtacctgcagaagccaggccagctctcaaaaactcctgatctacaaactttcaaccgattttctgggggtcccaga
 caggttcagtggtcagtggtcagggacagatttcacactcaagatcagcagagtgagggtgaggatctgggagttatttctgctc
 tcaaagtacacatgttccgtggacgttcggtggaggcaccagctggaaatcaaagatggcggtggctcggcggtggtggatct
 ggaggaggtgggagctctcagattactctgaaagagtctggcctgggatcttgcagccctcccagaccctcagctgacttgttctt
 35 tctctgggttttactgaccacttatggtataggagtgggttgattcgtcagcctccagggaagggtctggagtggctgacacacat
 ttggtggaatgataataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcct
 caagatcgccaatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctggtca
 ctgtctctgctgatca

Amino acid sequence:

40 MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
 HWYLQKPGQSPKLLIYKLSNRFSGVSPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
 QSTHVPWTFGGGTKLEIKDGGGSGGGGSGGGGSSQITLKESGPGILQPSQTLSTCS
 FSGFSLTTYGIGVGWIRQPPGKGLEWLTHIWWNDNKYYNTALRSRLTISKDSSNN
 45 QVLLKIANVDTADTATYYCLYGYTYWGQGLVTVSAD

65. UCHL-1 VH I11SL12S

Nucleotide sequence:

50 gggagctctcagattactctgaaagagtctggccctgggatcttgcagccctcccagaccctcagctgacttgttcttctctgggtt
 tctactgaccacttatggtataggagtaggttgattcgtcagcctccagggaagggtctggagtggctgacacacatttgggtgaat

gataataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcctcaagatcgc
caatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggaactctggctactgtctctgct
gatca

- 5 Amino acid sequence:
(GSS)QITLKESGPGSSQPSQTLSTCSFSGFSLTTYGIGVGWIRQPPGKGLEWLTHIW
WNDNKYYNTALRSRLTISKDSSNNQVLLKIANVDTADTATYYCLYGYTYWGQGT
LTVTSAD

10

66. UCHL-1 scFv VH L11S

Nucleotide sequence:

- gttgtaagcttgcgcctgaagttgcctgtaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgaccc
aaactccactctccctgctgtcagcttggagatcaggcctccatctcttcagatctagtcagagccttcttacagtaaatgaaac
15 acctatttacattggtacctgcagaagccaggccagctcctcaaaactcctgatctacaaactttccaaccgattttctgggggtccaga
cagggtcagtgaggatggatcaggagacagattcacactcaagatcagcagagtgagggtgaggatctgggagtttattctgctc
tcaaagtacacatgtccgtggacgttcggtggaggcaccaagctggaaatcaaagatggcgggtggctcgggcgggtggatct
ggaggaggtgggagctctcagattactctgaaagagtctggcctgggagctcccagccctcccagaccctcagctgactgttct
tttctctgggttttactgaccacttatggtataggagtaggttgattcgtcagcctccagggaagggtctggagtggctgacacac
20 atttggtggaatgataataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactc
ctcaagatcgccaatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggaactctggct
actgtctctgctgatca

Amino acid sequence:

- 25 MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
HWYLQKPGQSPKLLIYKLSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
QSTHVPWTFGGGTKLEIKDGGGSGGGGSGGGGSSQITLKESGPGSSQPSQTLSTC
SFGFSLTTYGIGVGWIRQPPGKGLEWLTHIWWNDNKYYNTALRSRLTISKDSSNN
QVLLKIANVDTADTATYYCLYGYTYWGQGT LTVTSAD

30

67. UCHL-1 scFv (SSS-S)H WCH2 WCH3

Nucleotide sequence:

- gttgtaagcttgcgcctgaagttgcctgtaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgaccc
35 aaactccactctccctgctgtcagcttggagatcaggcctccatctcttcagatctagtcagagccttcttacagtaaatgaaac
acctatttacattggtacctgcagaagccaggccagctcctcaaaactcctgatctacaaactttccaaccgattttctgggggtccaga
cagggtcagtgaggatggatcaggagacagattcacactcaagatcagcagagtgagggtgaggatctgggagtttattctgctc
tcaaagtacacatgtccgtggacgttcggtggaggcaccaagctggaaatcaaagatggcgggtggctcgggcgggtggatct
ggaggaggtgggagctctcagattactctgaaagagtctggcctgggattctcagccctcccagaccctcagctgactgttctt
40 tctctgggttttactgaccacttatggtataggagtaggttgattcgtcagcctccagggaagggtctggagtggctgacacacat
ttggtggaatgataataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcct
caagatcgccaatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggaactctgtca
ctgtctctgctgatcaggagcccaaatctctgacaaaactcacacatcccaccgtctcagcacctgaactcctgggtggaccgt
cagttctctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtggacgtgagc
45 cacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca
gtacaacagcacgtaccgtgtggtcagcgtctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtcaaggct
tccaacaaagccctcccagccccatcgagaaaaccatctccaagccaaagggcagccccgagaaccacaggtgtacacct
gccccatcccggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaaggcttctatccaagcgacatcgccgtg
gagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctac
50 agcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctctcatgctccgtgatgcatgaggctctgcacaaccact
acacgcagaagagcctctcctgtctccgggtaaatgatctaga

Amino acid sequence:

MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
 HWYLQKPGQSPKLLIYKLSNRFSGVSPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
 5 QSTHVPWTFGGGTKLEIKDGGGSGGGGSGGGGSSQITLKESGPGILQPSQTLSTLCS
 FSGFSLTTYGIGVGWIRQPPGKGLEWLTHIWWNDNKYYNTALRSRLTISKDSSNN
 QVLLKIANVDTADTATYYCLYGYTYWGQGLVTVSADQEPKSSDKTHTSPSSAP
 ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 10 PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
 DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

68. UCHL-1 scFv VHL11S (SSS-S)H WCH2 WCH3

15 Nucleotide sequence:

ggtgtaagcttgccgcatgaagtgccgtgtaggctgttggtgctgatgttctggattcctgctccatcagtgatgttgatgaccc
 aaactccactctccctgctgagtcagtgatcagccctccatctctgagatcagtcagagcctctttacagtaaggaaac
 acctattacattggtacgtgcagaagccaggcagtcctcaaaactcctgatctacaaactttccaaccgattttctgggggtccaga
 cagggtcagtggtgagtcagggacagatttcacactcaagtcagcagagtgagggtgaggatctgggagttatttctgctc
 20 tcaaagtacacatgtccgtggacgttcggtggagccaccaagctggaatcaaagatggcggtggtcggcggtggtgagtc
 ggaggaggtgggagctcagattactctgaaagagtcgtggcctgggagctccagccctccagaccctcagtcgactgttc
 ttctctgggttttactgaccacttatggtataggagtaggttggttcgagcctccagggaagggtctggagtggtgacacac
 atttggtggaatgataataagtactataacacagccctgaggagccggtcacaatctcaaggattcctcaacaaccaagtactc
 ctcaagatcgccaatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggtggtc
 25 actgtctctgctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggtggaccg
 tcagtctctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgagggtcacatgcgtggtggtggacgtgag
 ccacgaagaccctgagggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccagacaaagccgcgggaggagc
 agtacaacagcacgtaccgtgtggtcagcgtctcaccgtctgaccagagactggtgaatggcaaggagtacaagtgaaggt
 ctccaacaaagccctccagccccatcgagaaaacatctccaaagccaaagggcagcccgagaaccacaggtgtacacc
 30 tgcccccacccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccaagcgacatcgccgt
 ggagtgaggagagcaatgggcagccgggagaaactacaagaccacgcctcccgtgctggactccgacggctccttctctcta
 cagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacca
 ctacacgcagaagagcctctccctgtctccgggtaaatgatagaa

35 Amino acid sequence:

MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
 HWYLQKPGQSPKLLIYKLSNRFSGVSPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
 QSTHVPWTFGGGTKLEIKDGGGSGGGGSGGGGSSQITLKESGPGSSQPSQTLSTLCS
 40 SFGFSLTTYGIGVGWIRQPPGKGLEWLTHIWWNDNKYYNTALRSRLTISKDSSNN
 QVLLKIANVDTADTATYYCLYGYTYWGQGLVTVSADQEPKSSDKTHTSPSSAP
 ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
 45 DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

69. 5B9 VH L11S

Nucleotide sequence:

gggagctctcaggtgcagctgaagcagtcaggacctggctcagtcagtcctcacagagcctgtccatcacctgcacagtctctg
 gtttctcattaactacatgctgtactggttcgccagtcctcaggaaagggtctggagtggtgggagtgatggagtggtgg
 50 aatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttcttaaaatgaacagt

tgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa
ggaacctcagtcaccgtctcctcag

Amino acid sequence:

5 (GSS)QVQLKQSGPGSVQSSQSLSTCTVSGFSLTTYAVHWVRQSPGKGLEWLGV
WSGGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIYYCARNGGDNYPYY
YAMDYWGQGTSTVTVSS

10 **73. 5B9 VH L11S scFv**

Nucleotide sequence:

aagcttgccgcatgaggttctctgctcagcttctgggggtgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgattggtatctgcagaagccaggccagctcctcagctcctgattatcagatgtccaaccttgccctcaggagtcagcagaca
15 ggctcagtagcagtgaggctcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcgggtgctgggaccaagctggagctgaaacgggggtggcgggtggctcgggcgggtgggtgggt
cgggtggcggcgggagctctcaggtgcagctgaagcagtcaggacctggctcagtcagtcctcacagagcctgtccatcacct
gcacagctctctggttctcattaactacctatgctgtacactgggttcgccagctctccaggaaagggtctggagtgggtgggagtgat
atggagtgggtggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttcttt
20 aaaatgaacagctctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
ctactgggggtcaaggaaacctcagtcaccgtctcctcag

Amino acid sequence:

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHLSNGITY
25 LYWYLQKPGQSPQLLIYQMSNLASGVDPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGSGGGGSGGGGSSQVQLKQSGPGSVQSSQSLSI
TCTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKS
QVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSTVTVSS

30

70. 5B9 scFv VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgccgcatgaggttctctgctcagcttctgggggtgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
35 cttatttgattggtatctgcagaagccaggccagctcctcagctcctgattatcagatgtccaaccttgccctcaggagtcagcagaca
ggctcagtagcagtgaggctcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcgggtgctgggaccaagctggagctgaaacgggggtggcgggtggctcgggcgggtgggtgggt
cgggtggcggcgggagctctcaggtgcagctgaagcagtcaggacctggctcagtcagtcctcacagagcctgtccatcacct
gcacagctctctggttctcattaactacctatgctgtacactgggttcgccagctctccaggaaagggtctggagtgggtgggagtgat
40 atggagtgggtggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttcttt
aaaatgaacagctctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
ctactgggggtcaaggaaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctc
agcacctgaactcctgggtggaccgtcagcttctcctctccccccaaaacccaaggacaccctcatgatctccggacccttgagg
tcacatgcgtgggtgggtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgc
45 caagacaaagccgcgggaggagcagtaacagcacgtaccgtgtgggtcagcgtcctcaccgtctgcaccaggactgggtga
alggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcag
ccccgagaaccacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtgctcaa
ggcttctatccaagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccggtgct
ggactccgacggctccttctctctacagcaagctaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctcc
50 gtgatgcatgaggctctgcacaaccactacacgcagaagagcctctcctctgtccgggtaaatgatctagag

Amino acid sequence:

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHNSGITY
 LYWYLQKPGQSPQLLIYQMSNLAGVDPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
 AQNLELPLTFGAGTKLELKRGGGGSGGGGSSQVQLKQSGPGSVQSSQSLSI
 5 TCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSKS
 QVFFKMNSLQPNDAIYYCARNGGDNPYYYYAMDYWGQGTSTVTVSSDQEPKSS
 DKTHTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
 YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
 PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
 10 ENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS
 LSPGK

15 **76. 2H7 scFv VH L11S (SSS-S)H P238SCH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggatttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 cccagctctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttgaggccagctcaagtgttaagttacatgcact
 ggtaccagcagaagccaggatcctccccaaacctggatttatccccatccaacctggcttctggagtcctgctcgttcagtg
 20 gcagtggtctgggacctctactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggc
 tacacattaccagttacaatatgactgggtaagcagacacctagacaggcctggaatggattggagctattatccaggaaat
 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 25 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttctgctgtgggcac
 agggaccacggtcaccgtctctctgacagaggccaaatcttctgacaaaactacacatccccaccgtcctcagcaccatgaact
 cctgggggggacgtcagcttctctctcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgctgtg
 tgggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc
 cgcgggaggagcagtaaacagcagcgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
 30 tacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaacc
 acaggtgtacacctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtgcaaggttctatccc
 agcgacatcgccgtggagtgaggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctggactccgacg
 gctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgatgag
 gctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaatgatctaga

35

Amino acid sequence:

MDFQVQIFSLLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QKPGSSPKPWYAPS NLAGVDPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
 40 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 45 NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
 PGK

78. 2H7 scFv VH L11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

50 aagcttgccgccatggatttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 cccagctctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttgaggccagctcaagtgttaagttacatgcact

ggtaccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagt
 gcagtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
 5 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaat
 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcac
 agggaccacggtcaccgtcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaact
 cctgggggggaccgtcagcttcttcttccccccaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtgg
 10 tggaggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc
 cgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
 tacaagtgaaggcttccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaacc
 acaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccc
 agcgacatcgccgtggagtgggagagcaatgggcagccgggagaaactacaagaccacgcctcccgtgctggactccgacg
 15 gctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTTCRASSSVSYMHWY
 20 QQKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGSGGGGSGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDK
 THTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 25 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
 PGK

30

79. 2H7 scFv VH L11S (CSS-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgcgccatggatttcaagtgcagatttcaagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctct
 cccagctcagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcact
 35 ggtaccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagt
 gcagtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtg
 ggagctctcaggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
 tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaat
 40 ggtgatacttctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcac
 agggaccacggtcaccgtcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaact
 cctgggggggaccgtcagcttcttcttccccccaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtgg
 tggaggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc
 45 cgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
 tacaagtgaaggcttccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaacc
 acaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccc
 agcgacatcgccgtggagtgggagagcaatgggcagccgggagaaactacaagaccacgcctcccgtgctggactccgacg
 50 gctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QQKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAESVRPGASVKMSCK
 5 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSCDK
 THTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 10 NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
 PGK

77. G8-1 scFv VHL11S (SCS-S)H WCH2 WCH3**Nucleotide sequence:**

15 gttgtaagcttgccgcatggatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagat
 gactcagctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttgg
 cttggtatcagcagaaacagggaatactcctcagctcctgtctcttttgcataaaccttagcagaaggtgtgcatcaaggttcag
 tggcagtggtacaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatcattccg
 ataatccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcgggcgggtggtgggtcgggtggcggc
 20 ggatcgtcagcgggtccagctgcagcagcttgacctgagtcggaaagcctggcgcttcagtgaaagtcttctgcaaggcttctgg
 ttactcattcactggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtgattggaaatattgacatttattatg
 gtgtactacctacaaccggaagtcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaa
 gactctgacatctgaggactctgcagctctattactgtgaagatcggtcggcctatggactactgggtgaaggaacctcagtcac
 cgtctcttctgatcaggagcccaatcttctgacaaaactcacacatgccaccgctcctcagcacctgaacacctgggtggaccgtc
 25 agtcttctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtgggtggacgtgagcc
 acgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca
 gtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggct
 tccaacaaagccctccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacct
 gccccatccgggatgagctgaccaagaaccaggtcagcctgacctgctgctgcaaggcttctatccaagcgacatcgccgtg
 30 gactgggagagcaatgggcagccggagaacaactacaagaccacgcctccgtgctggactccgacgggtccttcttctctac
 agcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctatgctccgtgatgcatgaggctctgcacaaccact
 acacgcagaagagcctctcctgtctcgggtaaatgatctagag

Amino acid sequence:

35 MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLA
 YQQKQKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLPEDSGSYFCQHHS
 DNPWTFGGGTELEIKGGGSGGGGSGGGSSAVQLQQSGPESEKPGASVKISCKA
 SGYSFTGYNMNWKQNNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
 AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSTVTVSSDQEPKSSDKTHTCPPSS
 40 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
 GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
 VLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

45 79.. G28-1 scFv VHL11S (CCS-P)H WCH2 WCH3**Nucleotide sequence:**

gttgtaagcttgccgcatggatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagat
 gactcagctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttgg
 cttggtatcagcagaaacagggaatactcctcagctcctgtctcttttgcataaaccttagcagaaggtgtgcatcaaggttcag
 50 tggcagtggtacaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatcattccg
 ataatccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcgggcgggtggtgggtcgggtggcggc

ggatcgtcagcgggtccagctgcagcagcttgacctgagtcggaaaagcctggcgcttcagtgaagatttctgcaaggcttctgg
 ttactcattcactggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtgattggaaatattgatccttattatg
 gtgtactacctacaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaa
 5 gactctgacatctgaggactctgcagcttattactgtgcaagatcggcggccctatggactactgggtcaaggaacctcagtcac
 cgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatgtccaccgtccccagcacctgaactcctgggtggaccgtc
 agtcttctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtgggtggacgtgagcc
 acgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca
 gtacaacagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc
 10 tccaacaaagccctcccagcccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctt
 gccccatccccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccaagcgacatcgccgtg
 gactgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctac
 agcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccact
 acacgcagaagagcctctccctgtctccgggtaaatgatctaga

15 Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLA
 YQKQKQKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLOPEDSGSYFCQHHS
 DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
 SGYSFTGYNMNWKQNNGKSLEWIGNIDPYGGTTYNRKFKGKATLTVDKSSST
 20 AYMQLKSLTSEDSAVYYCARSVGPM DYWGQTSVTVSSDQEPKSCDKTHTCPPS
 PAPELLGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP
 25 PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

80. G28-1 scFv VH L11S (SCC-P)H WCH2 WCH3

Nucleotide sequence:

gttgtaagcttccgccatggtatccacagctcagttccttgggtgctgctgctgtggcttacaggtggcagatgtgacatccagat
 gactcagctctccagcctcctatctgcatctgtggagagactgtccatcacatgtcgaacaagtgaatgtttacagttatttgg
 30 cttggatcagcagaaacagggaatctcctcagctcctgtctcttttgcataaaccttagcagaaggtgtccatcaaggttcag
 tggcagtgatcaggcacacagtttcttgaagatcagcagcctgcagcctgaagattctggaagttatttctgcaacatcattccg
 ataatccgtggacgttcggtggagaccgaactggagatcaaaggtggcgggtggtcgggcgggtgggtgggcggc
 ggatcgtcagcgggtccagctgcagcagcttgacctgagtcggaaaagcctggcgttcagtgaagatttctgcaaggcttctgg
 ttactcattcactggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtgattggaaatattgatccttattatg
 35 gtgtactacctacaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaa
 gactctgacatctgaggactctgcagcttattactgtgcaagatcggcggccctatggactactgggtcaaggaacctcagtcac
 cgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatgccaccgtgccagcacctgaactcctgggtggaccgtc
 agtcttctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtgggtggacgtgagcc
 acgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca
 40 gtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc
 tccaacaaagccctcccagcccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctt
 gccccatccccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccaagcgacatcgccgtg
 gactgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctac
 agcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccact
 45 acacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLA
 YQKQKQKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLOPEDSGSYFCQHHS
 50 DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
 SGYSFTGYNMNWKQNNGKSLEWIGNIDPYGGTTYNRKFKGKATLTVDKSSST

AYMQLKSLTSEDSAVYYCARSVGPMDYWGGQTSVTVSSDQEPKSSDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
 KGQPREPVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
 5 PVLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

81. G28-1 scFv VH L11S mIgE CH2 CH3 CH4

Nucleotide sequence:

10 aagcttcccgcctatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
 agtctccagcctccctatctgcatctgtggagagactgtcaccatcacatgtcgaacaagtgaataatgtttacagttatttggttggt
 atcagcagaaacagggaataatctcctcagctcctggtctcttttgcataaaccttagcagaaggtgtgcatcaaggttcagtgga
 gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagttattctgtcaacatcattccgataat
 ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtggtgggtcgggtggcggcggt
 15 cgtcagcgggtccagctgcagcagcttgacctgagtcggaaaagcctggcgttcagtggaagttcctgcaaggcttctggttact
 cattcactggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggaattggaatatgatccttattatggtggt
 actacctacaaccggaagttcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctc
 gacatctgaggactctgcagctattactgtgcaagatcggtcggccctatggactactgggtgcaaggaacctcagtcaccgtctc
 ttctgatcacgttcgacctgtcaacatcactgagcccacctggagctactccattcatcctgcgaccccaatgcattccactccacca
 20 tccagctgtactgcttcatttatggccacatcctaaatgatgtctctgtcagctggctaattggacgatcgggagataactgatacacttg
 cacaaactgttctaatacaggaggaaggcaaaactagcctctacctgcagtaaaactcaacatcactgagcagcaatggatgtctgaa
 agcaccttcacctgcaaggtcacctcccaaggcgtgactatttggccacactcggagatgccagatcatgagccacgggggtg
 tgattacctatctgatccaccagccccctggacctgtatcaaacgggtgtcccaagcttacctgtctggtggtggacctggaaa
 gcgagaagaatgtcaatgtgacgtggaaccaagagaagaagacttcagctcagcatccagtggtacactaagcaccacaataa
 25 cgccacaactagatcacctccatcctgcctgtagttgccaaggactggattgaaggctacggctatcagtgcatagtggaccaccc
 tgattttcccaagccattgtgcgttccatcaccaagaacccaggccagcgtcagccccgaggtatatgtgtccaccaccaga
 ggaggagagcaggagacaaacgcacactcacctgtttgatccagaactcttccctgaggatatctctgtgcagtggtggtgggatg
 gcaaactgatctcaaacagccagcacagtagcacacacccctgaaatccaatggctccaatcaaggcttcttcatcttcagtcgcc
 tagaggtcgccaagacactctggacacagagaaaacagttcacctgccaagtgtatcatgaggcacttcagaaacccaggaaaact
 30 ggagaaaacaatatccacaagccttggaacacctccctccgtccatcctagtaatatagagg

Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
 YQQKQKGKSPQLLVSFATLAEGVPSRFSGSGSGTQFSLKISSLOPEDSGSYFCQHHS
 35 DNPWTFGGGTELEIKGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
 SGYSFTGYNMNWNVQNNNGKSLEWIGNIDPYGGTTYNRKFKGKATLTVDKSSST
 AYMQLKSLTSEDSAVYYCARSVGPMDYWGGQTSVTVSSDHVRPNITEPTLELLH
 SSCDPNFAFHSTIQLYCFIYGHILNDVSVSWLMDDREITDTLAQTVLIKEEGLASTC
 SKLNITEQQWMSESTFTCKVTSQGVLDYLAHTRRCPDHEPRGVITYLPPSPLDLYQ
 40 NGAPKLTCLVVDLESEKNVNVTWNQEKTSVSASQWYTKHHNNATTSTISILPVV
 AKDWIEGYGYQCIVDHPDFPKPIVRSITKTPGQRSAPENVYVFPPEEESDKRTLTC
 LIQNFFPEDISVQWLGDGKLISNSQHSTTTPLKSNGSNQGFFIFSRLEVAKTLWTQR
 KQFTCQVIHEALQKPRKLEKTISTSLGNTSLRPS

45

82. G28-1 scFv VH L11S mIgA WH WCH2 T4CH3

Nucleotide sequence:

aagcttcccgcctatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
 agtctccagcctccctatctgcatctgtggagagactgtcaccatcacatgtcgaacaagtgaataatgtttacagttatttggttggt
 50 atcagcagaaacagggaataatctcctcagctcctggtctcttttgcataaaccttagcagaaggtgtgcatcaaggttcagtgga
 gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagttattctgtcaacatcattccgataat

ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcgggcggtggtgggtcgggtggcgcggtat
 cgtcagcgggtccagctgcagcagcttgacctgagtcggaagcctggcgcttcagtgaagattcctgcaaggcttctggttact
 cattcactggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggttggaatattgatccttattatggtggt
 actacctacaaccggaagttcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtc
 5 gacatctgaggactctgcagcttattactgtgcaagatcggtcggccctatggactactgggtcaaggaacctcagtcaccgtctc
 ttctgatcacatctgttctcctactactcctcctccaccttctgccagcccagcctgtcactgcagcggccagctcttgaggacct
 gctcctgggttcagatgccagcatcacatgtactctgaatggcctgagagatcctgaggagctgtcttcacctgggagccctccac
 tgggaaggtatgcagtgacagaagaaagctgtgcagaattcctcggtgctacagtgtgtccagcgtcctgcctgggtgtgctgag
 cgctggaacagtggcgcatcattcaagtgcacagttaccatcctgagctgtacacctaactggcacaattgccaaagtcacagt
 10 aacaccttcccacccaggtccacctgtaccgccgctcgaggagctggccctgaatgagctcgtgtccctgacatgcctgg
 tgcgagctttcaacctaaagaagtgtggtgcgatggctgcagtgaaatgaggagctgtcccagaaagctacatgtgtttgag
 cccctaaaggagccaggcgaggagccaccacctggtgacaagcgtgtgtgtatcagctgaaatctggaacaggtg
 gaccagtactcctgcagtggtgggccacgagccttgccatgaacttcaccagaagaccatcgaccgtctgtcgggtaaaccca
 ccaatgtcagcgtgtctgtgatcatgtcagagggagattgataatctagat

15 Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
 YQKQKQKSPQLLVSFATLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
 DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
 20 SGYSFTGYNMNWKQNNKGSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
 AYMQLKSLTSEDSAVYYCARSVGPMDYWGQTSVTVSSDHICSPPTTPPPSCQPS
 LSLQRPALDLLLLGSDASITCTLNGLRDPEGA VFTWEPSTGKDAVQKKA VQNSCG
 CYSVSSVLPGCAERWNSGASFKCTVTHPESDTLTGTIAKVTVNTFPPQVHLLPSPSE
 ELALNELVSLTCLVRAFNPKEVLVRWLHGNEELSPESYLVFEPLKEPGEGETTYLV
 25 TSVLRVSAEIKQGDQYSCMVGHEALPMNFTQKTIDRLSGKPTNVSVSVIMSEGD

83. G28-1 scFv Vh L11S hIgE CH2 CH3 CH4

Nucleotide sequence:

aagcttgcggccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
 30 agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatgtttacagttatttgcttggt
 atcagcagaacagggaaaatcctcagctcctgtctctttgcaaaaaccttagcagaaggtgtccatcaaggctcagtgga
 gtggatcaggcacacagtttctgaagatcagcagcctgcagcctgaagattctggaagttattctgtcaacatcattccgataat
 ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcgggcggtggtgggtcgggtggcgcggtat
 35 cgtcagcgggtccagctgcagcagcttgacctgagtcggaagcctggcgcttcagtgaagattcctgcaaggcttctggttact
 cattcactggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggttggaatattgatccttattatggtggt
 actacctacaaccggaagttcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtc
 gacatctgaggactctgcagcttattactgtgcaagatcggtcggccctatggactactgggtcaaggaacctcagtcaccgtctc
 ttctgatcacgtctgtccagggaacttcaccccggccaccgtgaagatcttacagtcgtcctgcgacggcgggcgggcacttcccc
 40 cgaccatccagctcctgtgcctcgtcttggttacacccagggaactatcaacatcacctggctggaggacgggcaggtcatgga
 cgtggacttgtccaccgctctaccacgcaggaggtgagctggcctccacacaaagcgagctcacctcagccagaagcactg
 gctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccagaaggtgtgcagattccaac
 ccgagaggggtgagcgctacctaagccggccagcccgttcgacctgttcacccgaagtcgcccacgatcacctgtctgtgtg
 tggacctggcaccagcaaggggaccgtgaacctgacctggctccgggagcagtggaagcctgtgaaccactccaccagaaag
 45 gaggagaagcagcgcaatggcacgttaacctgcacgtccacctgccggtgggcacccgagactggatcgagggggagacct
 accagtgagggtgacccacccacctgcccaggggcctcatcggttcacgaccaagaccagcgcccgctgctgctgcccc
 ggaagtctatgcgttgcgacggcggagtgccggggagccgggacaagcgacccctgcctgcctgatccagaacttcagcc
 tgaggacatctcggtgcagtggtgcacaacgaggtgcagctcccgagcggcgccagcagcagcagcagcccgcaagacc
 aagggtccggcttctctgtcttcagccgctggaggtgaccagggccgaatgggagcagaagatgagttcatctgccgtgcag
 50 tccatgaggcagcagagccctcacagaccgtccagcgagcgggtgtctgtaaatccgggtaaatgataatctaga

Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
 YQQKQKGKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
 DNPWTFGGGTELEIKGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
 5 SGYSFTGYNMNWWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
 AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSTVTVSSDHVCSRDFTPPTVKIL
 QSSCDGGGHFPPTIQLLCLVSGYTPGTINTWLEDGQVMDVDLSTASTTQEGELAS
 TQSELTL SQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSA YLSRPSFD
 10 LFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLP
 VGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDK
 RTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRA
 WEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

15 84. **G28-1 scFv VH L11S hIgA WH WCH2 T4CH3**

Nucleotide sequence:

atggtatccacagctcagttccttggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactcagctccagcct
 ccctatctgcatctgtgggagagactgtcaccatcacatgtcgaaacagtgaaatgtttacagttatttgcttggtatcagcagaaa
 cagggaataatctctcagctcctggtctctttgcaaaaaccttagcagaaggtgtgccatcaagggtcagtggcagtggtacaggc
 20 acacagttttctctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtcaacatcattccgataatccgtggacgttc
 ggtggaggccaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtggcggcgatcgtcagcggtcc
 agctgcagcagcttgacctgagtcggaaaagcctggcgttcagtgaaatttctgcaaggcttctggttactcattcactggcta
 caatatgaactgggtgaagcagaataatggaagagccttgagtggttgaaatattgatccttattatgggtgactacctacaac
 cggaagtcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctgacatctgagg
 25 actctgcagctctattactgtcagatcggtcggccctatggactactgggtcaaggaacctcagtcaccgtctctctgatcagcc
 agttccctcaactccacctacccatctccctcaactccacctacccatctcctcatgctgccacccccgactgtcactgcaccga
 ccggccctcgaggacctgctcttaggttcagaagcgtcctcacgtgcacactgaccggcctgagagatgcctcaggtgtcacctt
 cacctggacgccctcaagtgggaagagcgtgttcaaggaccactgaccgtgacctctgtggctgctacagcgtgtccagtgct
 ctgccgggctgtgccgagccatggaacctgggaagacctcactgtcactgtgcctaccccgagtccaagaccccgctaaccg
 30 ccacctctcaaaatccggaacacattccggcccaggtccacctgctgccgccgctcgaggagctggccctgaacgag
 ctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgcccc
 gcgagaagtacgtgactgggcatccggcaggagcccagccagggcaccaccaccttgcgtgtgaccagcatactgcgcgtg
 gcagccgaggactggaagaagggggacaccttctcctgcatgggtgggccacgaggccctgccgtggccttcacacagaagac
 catcgaccgcttgccgggtaaacccacccatgtcaatgtgtctgtgtcatggcgagggtggactgataatctaga

Amino acid sequence:

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
 YQQKQKGKSPQLLVSF AKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
 DNPWTFGGGTELEIKGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
 40 SGYSFTGYNMNWWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
 AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSTVTVSSDQVPSTPPTPSTPPT
 PSPSCCHPRLSLHRPALED

85. **HD37 VL**

Nucleotide sequence:

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggtccaggctccactggtgacattgtgctgacc
 caatctccagcttcttggtctgtctcttagggcagagggccacatctcctgcaaggccagccaaagtgttgattatgatggtgata
 gttatttgaactggtaccaacagattccaggacagccacccaactcctcatctatgatgcacatctagtttctgggatccacccc
 aggtttagtggtcagtggtgctgggacagacttcacctcaacatccatcctgtggagaagggtggatgctgcaacctatcactgtcag
 50 caaagtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaagggtggcgggtggctcgggcgggtgggtg
 gggtggcggcgggagctcg

Amino acid sequence:

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC
5 QQSTEDPWTFGGGTKLEIKGGGGSGGGGSGGGGSS

86. HD37 VH

Nucleotide sequence:

gggagctcgcaggttcagctgcagcagctctggggctgagctggtgaggcctgggtcctcagtgagatttctgcaaggcttctg
10 gctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggcttgagtgattggacagattggcctgga
gatggtgataactacaatggaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaact
cagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence:

(GSS)QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQI
WPGDGDNTYNGKFKGKATLTADESSSTAYMQLSSLASEDSA VYFCARRETTTVG
15 RYYYAMDYWGQGTSTVTVSSDQ

87. HD37 scFv

Nucleotide sequence:

aagcttgcgcagcatggagacagacacactcctgctatgggtgctgctgctctgggtccaggctccactggtgacattgtgctgacc
caatctccagcttcttggctgtgtcttagggcagagggccaccatctcctgcaaggccagccaaagtgtgattatgatggtgata
gttatttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcacccaatctagtttctgggatccaccc
25 aggtttagtggcagtggtgctgggacagacttcacctcaacatccatcctgtggagaagggtgatgctgcaacctatcactgtcag
caaagtactgaggatccgtggacgttcggtggaggcaccaagctggaatcaaagggtggcgggtggcggcggtggtgggtc
gggtggcggcggtatgacaggttcagctgcagcagctctggggctgagctggtgaggcctgggtcctcagtgagatttctgc
aaggcttctggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggcttgagtgaltggacagatt
tggcctggagatggtgataactacaatggaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagccta
30 catgcaactcagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattacta
tgctatggactactggggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence:

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
35 YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC
QQSTEDPWTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLQQSGAELVRPGSSVKI
SCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDNTYNGKFKGKATLTAD
ESSSTAYMQLSSLASEDSA VYFCARRETTTVGRYYYAMDYWGQGTSTVTVSSDQ

88. HD37 VHL11S:

Nucleotide sequence:

gggagctcgcaggttcagctgcagcagctctggggctgagtcggtgaggcctgggtcctcagtgagatttctgcaaggcttctg
gctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggcttgagtgattggacagattggcctgga
45 gatggtgataactacaatggaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaact
cagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence:

(GSS)QVQLQQSGAESVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQI
50 WPGDGDNTYNGKFKGKATLTADESSSTAYMQLSSLASEDSA VYFCARRETTTVG
RYYYAMDYWGQGTSTVTVSSDQ

89. HD37 scFv VHL11S:

Nucleotide sequence:

5 Aagcttgccgcatggagacagacacactcctgctatgggtgctgctgctctgggtccaggctccactggtgacattgtgctgac
 ccaatctccagcttcttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgtgattatgatggat
 agttattgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagttctgggatccacc
 cagggttagtggcagtggtctgggacagacttcaccctcaacatccatcctgtggagaagggtggatgctgcaacctatcactgtca
 gcaaagtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaagggtggcgggtggctcgggcgggtgggt
 10 cgggtggcggcgggagctcgcaggttcagctgcagcagctctggggctgagtcggtgaggcctgggtcctcagtgaagatttct
 gcaaggcttctggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggcttctgagtgattggacag
 atttggcctggagatggtgataactacaatggaaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagc
 ctacatgcaactcagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggcccgttatta
 ctatgctatggactactgggtcaaggaaacctcagtcaccgtctcctctgatcag

15 Amino acid sequence:

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
 YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAAITYHC
 QQSTEDPWTFGGGKLEIKGGGSGGGGSGGGGSSQVQLQQSGAESVRPGSSVKI
 20 SCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTAD
 ESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYAMDYWGQGTSTVTVSSDQ

90. HD37 scFv IgAH hIgG1 WCH2 T4CH3

Nucleotide sequence

25 aagcttgccgcatggagacagacacactcctgctatgggtgctgctgctctgggtccaggctccactggtgacattgtgctgacc
 caatctccagcttcttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgtgattatgatggtgala
 gtlatttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagttctgggatccaccc
 aggttagtggcagtggtctgggacagacttcaccctcaacatccatcctgtggagaagggtggatgctgcaacctatcactgtcag
 caaagtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaagggtggcgggtggctcgggcgggtgggtggtc
 30 ggggtggcggcgggagctcgcaggttcagctgcagcagctctggggctgagtcggtgaggcctgggtcctcagtgaagatttctg
 caaggcttctggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggcttctgagtgattggacaga
 tttggcctggagatggtgataactacaatggaaagttcaagggtaaagccactctgactgcagacgaatcctccagcacagcct
 acatgcaactcagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggcccgttattact
 atgctatggactactgggtcaaggaaacctcagtcaccgtctcctctgatcagccagttccctcaactccacctaccccatctccctc
 aactccacctaccccatctccctcatgctgccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcaga
 35 agcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgct
 gttaaggaccacctgaccgtgacctctgtggctgtacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatg
 ggaagaccttcactgtcactgtgcctaccccgagtccaagaccccgtaaccgccacccctctaaaatccggaacacattccg
 gcccaggtccacctgctgccgcccgctggaggagctggcctgaacgagctggtgacgctgacgtgcctggcacgtggct
 tcagccccaaggatgtgctggtcgtggtgctgaggggtcacaggagctgccccgcgagaagtacctgactgggcatccggc
 40 aggagcccagccagggcaccaccacctcgtgtgaccagcactactgcgcgtggcagccgaggactggaagaagggggacac
 cttctcctgcatggtgggcccagaggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccat
 gtcaatgtgtctgtgtcatggcggagggtggactgataatctaga

Amino acid sequence

45 METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
 YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAAITYHC
 QQSTEDPWTFGGGKLEIKGGGSGGGGSGGGGSSQVQLQQSGAESVRPGSSVKI
 SCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTAD
 ESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYAMDYWGQGTSTVTVSSDQ
 50 VPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTF
 WTPSSGKSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFCTAAYPESKTPLT

ATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP
REKYLTWASRQEPSQGTTFVAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQK
TIDRLAGKPTHVNVSVVMAEVD

5 91. HD37 scFv (SSS-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggtccaggctccactggtgacattgtgctgacc
caatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgtgattatgatggtgata
gttatttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatccaccc
10 aggttttagtggcagtggtctgggacagacttcacctcaacatccatcctgtggagaagggtgatgctgcaacctatcactgtcag
caaagtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaagggtggcgggtggtcggggcgggtggtgggtc
gggtggcggcggtatgtcacaggttcagctgcagcagctctggggctgagctggtgaggcctgggtcctcagtgaaatttctgc
aagccttctggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtggattggacagatt
tggcctggagatggtgataactacaatggaaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagccta
15 catgcaactcagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattacta
tgctatggactactggggtaaggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatcccc
accgtcctcagcacctgaactcctgggtggaccgtcagcttctcctctccccccaaaaccaaggacacctcatgatctcccgac
ccctgaggtcacatgcgtgggtgggtggacgtgagccacgaagaccctgaggtcaagttcaactggtagctggacggcggtggaggt
gcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccagg
20 actggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcgagaaaaccatctccaaagcca
aagggcagccccgagaaccacaggtgtacaccctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgc
ctggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcc
tcccgctgctggactccgacggctccttctcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct
catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctcctctgtcctcggttaafgatctaga
25

Amino acid sequence:

METDTLLLWVLLLWVPGSTGDIVLTQSPASLA VSLGQRATISCKASQSVDYDGDS
YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC
QQSTEDPWTFGGGGTKLEIKGGGGSGGGGSGGGGSSQVQLQQSGAELVRPGSSVKI
30 SCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTAD
ESSSTAYMQLSSLASEDSA VYFCARRETTTVGRYYYAMDYWGQGTSTVTVSSDQE
PKSSDKTHTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
35 NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

92. HD37 scFv VH L11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggtccaggctccactggtgacattgtgctgacc
caatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgtgattatgatggtgata
gttatttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatccaccc
aggttttagtggcagtggtctgggacagacttcacctcaacatccatcctgtggagaagggtgatgctgcaacctatcactgtcag
45 caaagtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaagggtggcgggtggtcggggcgggtggtgggtc
gggtggcggcggtgagctgcaggttcagctgcagcagctctggggctgagtcggtgaggcctgggtcctcagtgaaatttctg
caaggcttctggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtggattggacaga
tttggcctggagatggtgataactacaatggaaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagcct
acatgcaactcagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattact
atgctatggactactggggtaaggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatccc
50 caccgtcctcagcacctgaactcctggggggaccgtcagcttctcctctccccccaaaaccaaggacacctcatgatctccggg
accctgaggtcacatgcgtgggtgggtggacgtgagccacgaagaccctgaggtcaagttcaactggtagctggacggcggtggag

gtgcataatgccaagacaaaagccgaggagcagtagacaacagcacgtaccgtgtggcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagcc
aaagggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctg
cctggcacaaggcttctatcccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgc
5 ctcctgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctt
ctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

METDTLLLWVLLLWVPGSTGDIVLTQSPASLA VSLGQRATISCKASQSVDYDGDS
10 YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNHPVEK VDAATYHC
QQSTEDPWTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLQQSGAESVRPGSSVKI
SCKASGYAFSSYWMNWVKQRPGGLEWIGQIWPGDGDTN YNGKFKGKATLTAD
ESSSTAYMQLSSLASEDSA VYFCARRETTTVGRYYYAMDYWGQGSVT VSSDQE
PKSSDKTHTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEV
15 KFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYT
QKSLSLSPGK

20 91. L6 VL

Nucleotide sequence:

atggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctccagctccag
caatcctgtctgcatctccaggggagaaggtcacattgactgcagggccagctcaagtgttaagtttcatgaactggtaccagcaga
agccaggatcctccccaaaccctggatttatgccacatccaatttgcttctgagttccctggcgccttcagtggcgagtggtctgg
25 gacctcttactctctcgcaatcagcagagtgagggtgaagatgctgccacttallactgccagcagtggaatagtaaccactcac
gttcggtgctgggaccaagctggagctgaaagagctccttggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcgat
ct

Amino acid sequence:

30 MDFQVQIFSLLISASVIMSRGQIVLSQSPAILSASPGEKVTLT CRASSSVSFMNWYQ
QKPGSSPKPWYIATSNLASEFPGRFSGEWSGTSYSLAISRVEAEDAATYYCQQWNS
NPLTFGAGTKLELKELSGGGGSGGGGSGGGGS

35 92. L6 VH

Nucleotide sequence:

cagatccagttgggtgcagcttgacctgagctgaagaagcctggagagacagtcagatctcctgcaaggcttctgggtatacctt
caciaactatggaatgaactgggtgaagcaggctccaggaaagggttaaaagtggatgggctggataaacacctacactggaca
gccaacatatgctgatgactcaagggacggttgccctctcttgaaacctctgcctacactgcctatttgcatcaacaacctca
aaaatgaggacatggctacatatctgtgcaagatttagctatgtaactcacgttacgctgactactggggccaaggcaccactct
40 cacagctcctctgatca

Amino acid sequence:

45 QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTY
TGQPTYADDFKGRFAFSLETSAYTAYLQINNLKNEDMATYFCARFSYGN SRYADY
WGQGTTTLTVSSD

50 93. L6 scFv

Nucleotide sequence:

atggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctccagctccag
caatcctgtctgcatctccaggggagaaggtcacattgactgcagggccagctcaagtgttaagtttcatgaactggtaccagcaga
agccaggatcctccccaaaccctggatttatgccacatccaatttgcttctgagttccctggcgccttcagtggcgagtggtctgg

gacctcttactctctcgcaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggaatagtaacccactcac
gttcgggtgctgggaccaagctggagctgaaagagctctctgggtggcggtggctcgggcgggtgggtgggtcgggtggcgggcgat
ctctgcagatccagttggtgcagctctggacctgagctgaagaagcctggagagacagtcaagatctctgcaaggcttctgggtat
accttcacaaactatggaatgaactgggtgaagcaggctccaggaaagggttaaaagtggatgggctggataaacacctactg
5 gacagccaacatatgctgatgacttcaagggacggtttgccttctcttggaaacctctgcctacactgcctatttgcagatcaacaac
ctcaaaaatgaggacatggctacatatcttctgtgcaagatttagctatggtaactcacgttacgctgactactggggccaaggcacca
ctctcacagtctctctgatca

Amino acid sequence:

10 MDFQVQIFSLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWWYQ
QKPGSSPKPWYATSNLASEFPGRFSGEWSGTSYSLAISRVEAEDAATYYCQQWNS
NPLTFGAGTKLELKELSGGGGSGGGGSGGGGSLQIQLVQSGPELKKPGETVKISCK
ASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGOPTYADDFKGRFAFSLETS
15 YTAYLQINNLKNEDMATYFCARFSYGNSRYADYWGQGTTTLTVSSD

94. L6 VHL11S

Nucleotide sequence:

ctgcagatccagttggtgcagctctggacctgagctgaagaagcctggagagacagtcaagatctctgcaaggcttctgggtatac
cttcacaaactatggaatgaactgggtgaagcaggctccaggaaagggttaaaagtggatgggctggataaacacctactgga
20 cagccaacatatgctgatgacttcaagggacggtttgccttctcttggaaacctctgcctacactgcctatttgcagatcaacaacct
caaaaatgaggacatggctacatatcttctgtgcaagatttagctatggtaactcacgttacgctgactactggggccaaggcaccact
ctcacagtctctctgatca

Amino acid sequence:

25 QIQLVQSGPESKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTY
TGQPTYADDFKGRFAFSLETSAYTAYLQINNLKNEDMATYFCARFSYGNSRYADY
WGQGTTTLTVSSD

95. L6 VH L11S scFv

30 Nucleotide sequence;

Aagcttgttgtatggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggacaaattgttctctc
ccagctctccagcaatctgtctgcatctccaggggagaaggtcacattgacttgcagggccagctcaagtgttaagttcatgaactg
gtaccagcagaagccagatcctcccccacacctggattatgccacatccaatttggttctgagttccctggctgcttcagtggc
gagtggctctgggaccttctctcgaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggaatagt
35 aaccactcacgttcggtgctgggaccaagctggagctgaaagagctctctgggtggcggtggctcgggcgggtgggtgggtcgggt
ggcgggcgatctctgcagatccagttggtgcagctctggacctgagctgaagaagcctggagagacagtcaagatctctgcaag
gcttctgggtataccttcacaaactatggaatgaactgggtgaagcaggctccaggaaagggttaaaagtggatgggctggataaa
cacctacactggacagccaacatatgctgatgacttcaagggacggtttgccttctcttggaaacctctgcctacactgcctatttgc
agatcaacaacctcaaaaatgaggacatggctacatatcttctgtgcaagatttagctatggtaactcacgttacgctgactactgggg
40 ccaaggcaccactctcacagtctctctgatca

Amino acid sequence:

45 MDFQVQIFSLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWWYQ
QKPGSSPKPWYATSNLASEFPGRFSGEWSGTSYSLAISRVEAEDAATYYCQQWNS
NPLTFGAGTKLELKELSGGGGSGGGGSGGGGSLQIQLVQSGPESKKPGETVKISCK
ASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGOPTYADDFKGRFAFSLETS
YTAYLQINNLKNEDMATYFCARFSYGNSRYADYWGQGTTTLTVSSD

96. L6 Or L6 VHL11S scFv IgAH hIgG1 WCH2 WCH3

50 Nucleotide sequence: (L6 VHL11S is shown)

aagcttggtgtaaggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctcc
 cagctccagcaatcctgtctgcatctccaggggagaaggtcacattgacttgaggccagctcaagtgttaagttcatgaactgg
 taccagcagaagccaggatcctcccccaccctggatttatgccacatccaatttggtctctgagttccctggctgcttcagtggcg
 agtggtctgggacctcttactctctcgaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagta
 5 acccactcacgttcggtgctgggaccaagctggagctgaaagagctctctggtggcgggtggctcgggcgggtgggtgggtcgggtg
 gcggcggtatctctgcagatccagttggtgagctctggacctgagtcgaagaagcctggagagacagtcagatctcctgcaaggc
 ttctgggtataccttcacaaactatggaatgaactgggtgaagcaggctccaggaaagggtttaaagtggatgggctggataaaca
 cctacactggacagccaacatatgctgatgacttcaaggagcggtttgccttctctttgaaacctctgcctacactgcctatttgcag
 atcaacaacctcaaaaatgaggacatggctacatattctgtgcaagatttagctatgtaactcacgttacgctgactactggggcc
 10 aaggcaccactctcacagctcctctgatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctcc
 ctcatgcgacactgaactcctggggggaccgtcagttctctctccccccaaacccaaggacacctcatgatctcccggacc
 ctgaggtcacatgcgtgggtgggacgtgagccacgaagacctgaggtcaagttcaactgggtacgtggacggcgtggaggtgc
 ataatgccaagacaaagccgaggagagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggact
 ggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaag
 15 ggagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctg
 gtcaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcc
 cgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctctcat
 gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

20 Amino acid sequence:

MDFQVQIFSFLISASVIMSRGQIVLSQSPAILASPGKEKVTLTCSRASSSVSFMNWFYQ
 QKPGSSPKPWYATSNLASEFPGRFSGEWSGTSYSLAISRVEAEDAATYYCQQWNS
 NPLTFGAGTKLELKELSGGGGSGGGGSGGGGSLQIQLVQSGPESKKPGETVKISCK
 ASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTQPTYADDFKGRFAFSLETS
 25 YTAYLQINNKNEDMATYFCARFSYGNTRYADYWGQGTTLTVSSDQVPSTPPTP
 SPSTPPTPSPSCAPELLGGPSVFLFPPKPKDTLMISRTPVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTTPVLDSGFSFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSL
 30 SLSPGK

97. L6 scFv VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

35 aagcttggtgtaaggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctcc
 cagctccagcaatcctgtctgcatctccaggggagaaggtcacattgacttgaggccagctcaagtgttaagttcatgaactgg
 taccagcagaagccaggatcctcccccaccctggatttatgccacatccaatttggtctctgagttccctggctgcttcagtggcg
 agtggtctgggacctcttactctctcgaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagta
 acccactcacgttcggtgctgggaccaagctggagctgaaagagctctctggtggcgggtggctcgggcgggtgggtgggtcgggtg
 40 gcggcggtatctctgcagatccagttggtgagctctggacctgagtcgaagaagcctggagagacagtcagatctcctgcaaggc
 ttctgggtataccttcacaaactatggaatgaactgggtgaagcaggctccaggaaagggtttaaagtggatgggctggataaaca
 cctacactggacagccaacatatgctgatgacttcaaggagcggtttgccttctctttgaaacctctgcctacactgcctatttgcag
 atcaacaacctcaaaaatgaggacatggctacatattctgtgcaagatttagctatgtaactcacgttacgctgactactggggcc
 aaggcaccactctcacagctcctctgatcaggagcccaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaac
 45 tcttggggggaccgtcagttctcttccccccaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtg
 gtggtggacgtgagccacgaagacctgaggtcaagttcaactgggtacgtggacggcgtggaggtgcataatgccaagacaaag
 ccgaggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaagga
 gtacaagtgaaggtctccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
 cacaggtgtacacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaggcttctatcc
 50 cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccctgctggactccgac

ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

5 MDFQVQIFSLLISASVMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWWYQ
QKPGSSPKPWYATSNLASEFPGRFSGEWSGTSYSLAISRVEAEDAATYYCQQWNS
NPLTFGAGTKLELKELSGGGGSGGGGSGGGGSLQILVQSGPESKKPGETVKISCK
ASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGQPTYADDFKGRFAFSLETS
10 YTAYLQINNLKNEDMATYFCARFSYGNRYADYWGQGTTLTVSSDQEPKSSDKT
HTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
15 NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

98. P238 CH2 WCH3

a. P238S CH2 WCH3

Nucleotide sequence:

20 cctgaactcctggggggatcgtcagttcttcttcccccaaaaaccaaggacaccctcatgatctcccgaccctgaggtcac
atgctggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaa
gacaaagccgcgggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaaggcagccc
cgagaaccacaggtgtacaccctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgctgtgtaaagg
25 cttctatcccagcgacatcgccgtggagtgggagagcaatggcgagccggagaacaactacaagaccacgcctcccgtgtgg
actccgacggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtg
atgatgaggtctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

30 PELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
LDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

35 b. (SSS-S)H P238S CH2 WCH3

Nucleotide sequence:

tgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggatcgtcagttcttct
cttcccccaaaaaccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacgaaga
ccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaaca
40 gcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaaca
agccctcccagccccatcgagaaaacaatctccaaagccaaaggcgagccccgagaaccacaggtgtacaccctgccccat
cccggtatgagctgaccaagaaccaggtcagcctgacctgctgtgtaaaggcttctatcccagcgacatcgccgtggagtggg
agagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtgactccgacggctccttcttctctacagcaagct
caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacga
45 gaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

50 DQEPKSSDKTHTSPSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE

WESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK*

99. a.CD16-6 low (ED) +NL+ (SSS-S)H P238S CH2 WCH3

5 Nucleotide sequence:

ggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggatcgtcagcttctcttccc
cccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgctggtggtggacgtgagccacgaagaccctga
ggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgt
accgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgcagggtctccaacaaagccct
10 cccagcccccacatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatccggg
atgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtggagtgggagagca
atgggcagccggagaacaactacaagaccacgcctcccgctggtgactccgacggctccttctctctacagcaagctcaccgt
ggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagag
cctctccctgtctccgggtaaatgatctaga

15

Amino acid sequence:

MWQLLLPTALLLVLSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPE
DNSTQWFHNESSLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLL
LQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATL
20 KDSGSYFCRGLVGSKNVSETVNITITQGLADQEPKSSDKTHTSPSSAPELLGGSS
VFLFPPKPKDTLMISRTPVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

25

100. b.CD16-6 low (ED)+HE4LP+(SSS-S)H P238S CH2 WCH3

Nucleotide sequence:

aagcttgccgcatgctgctgtgcctaggcccgctagccgccccctcctcagcctgctgctgttcggcttcaccctagtct
caggcacccggtgcaatcggactgaagatctccaaaggctgtggtgttctggagcctcaatggtacagggtgctcgagaagg
30 acagtgtgactctgaagtgccaggaggcctactccctgaggacaattccacacagtgtttcacaatgagagcctcatctcaagc
caggcctcgagctacttcattgacgtgccacagtcgacgacagtggagagtacaggtgccagacaaacctctccaccctcagtg
accgggtgcagctagaagtccatcggctgggtgttgcctcaggccccctcggtgggtgttcaaggagggaagaccctattcacctg
aggtgtcacagctggaagaacactgctctgcataaggtcacatattacagaatggcaaaggcaggaagtattttcatcataattctg
acttctacattccaaaagccacactcaaagacagcggctcctactctgcagggggcttgttgggagtaaaaatgtgtcttcagaga
35 ctgtgaacatcaccatcactcaaggtttggtgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcac
ctgaactcctggggggatcgtcagcttctcttccccccaaacccaaggacaccctcatgatctcccgaccctgaggtcacat
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaaga
caaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggc
aaggagtacaagtgaaggctctccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccg
40 agaaccacaggtgtacaccctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggctt
ctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggact
ccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatg
catgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctagaaa

45 Amino acid sequence:

MPACRLGPLAAALLLSLLLFGLTLVSGTGAMRTEDLPKAVVFLEPQWYRVLEKDS
VTLKCQGAYSPEDNSTQWFHNESSLISSQASSYFIDAATVDDSGEYRCQTNLSTLSD
PVQLEVHIGWLLQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFH
HNSDFYIPKATLKDSGSYFCRGLVGSKNVSETVNITITQGLADQEPKSSDKTHTSP
50 PSSAPELLGGSSVFLFPPKPKDTLMISRTPVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK

AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

101. CD16-9 high (ED) (SSS-S)H P238S CH2 CH3

5 a. CD16-9 high (ED)NL+(SSS-S)H P238S CH2 CH3

Nucleotide sequence:

gttgtaagcttccgccatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgcggactgaagatctccc
aaaggctgtggtgttcttgaggcctcaatggtacaggggtgctcgagaaggacagtgtgactctgaagtccaggaggcctactcc
cctgaggacaattccacacagtgtgttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgtgccacagtc
10 gacgacagtggagagtacaggtgccagacaaacctctccaccctcagtgacccggtgcagctagaagtccatcgtggtggtgt
tgctccaggccctcggtgggtgttcaaggagggaagacctattcacctgaggtgtcacagctggaagaacactgctctgcataag
gtcacatatttacagaatggcaaggcaggaagtattttcatcataattctgacttctacattccaaaagccacactcaaagacagcg
gctcctacttctgcagggggcttttgggagtaaaatgtgtcttcagagactgtgaacatcaccatcactcaagggttggtgatcag
gagcccaaatctctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggatcgtcagtcttctcttcccc
15 ccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtgagccacgaagacctgag
gtcaagttcaactggtacgtggacggcgtggagggtgcataatgccaagacaaagccgaggaggagcagtaaacagcacgta
ccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaagggtctccaacaagccctc
ccagccccatcgagaaaacaatctccaaagccaaaggcagccccgagaaccacaggtgtacacctgcccccatcccgga
tgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaat
20 gggcagccggagaacaactacaagaccacgcctcccggtggtgactccgacggctccttctctctacagcaagctcaccgtgg
acaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcc
tctccctgtctccgggtaaatgatctagaaa

Amino acid sequence:

25 MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCGAYSPE
DNSTQWFHNESSLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLL
LQAPRWVFKKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATL
KDSGSYFCRGLFGSKNVSETVNITITQGLADQEPKSSDKTHTSPSSAPELLGSS
VFLFPPKPKDTLMISRTPVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
30 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

CD16-9 high (ED)+HE4LP+hIgG1 (SSS-S)H P238S CH2 CH3

35 Nucleotide sequence:

aagcttccgccatgcctgctgtgcctaggcccgctagccgcgcctcctcctcagcctgctgctgttcggcttcaccctagtct
caggcaccggtgcaatgcggactgaagatctccaaaggctgtggtgttcttgaggcctcaatggtacaggggtgctcgagaagg
acagtgtgactctgaagtccaggaggcctactccctgaggacaattccacacagtgtgttcacaatgagagcctcatctcaagc
caggcctcgagctacttcattgacgtgccacagtcgacgacagtgagagtagcaggtgccagacaaacctctccaccctcagtg
40 acccggtgcagctagaagtccatcgtggtgtgtgtcctcaggccctcggtgggtgttcaaggagggaagacctattcacctg
aggtgtcacagctggaagaacactgctctgcataagggtcacatatttacagaatggcaaggcaggaagtattttcatcataattctg
acttctacattccaaaagccacactcaaagacagcggctcctacttctgcagggggcttttgggagtaaaatgtgtcttcagagac
tgtgaacatcaccatcactcaagggttggtgatcaggagcccaatcttctgacaaaactcacacatccccaccgtcctcagcacct
gaactcctggggggatcgtcagtcttctcttcccccaaaaccaaggacacctcatgatctccggaccttgagggtcacatg
45 cgtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgaggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggca
aggagtacaagtgaagggtcacaacaagccctccagccccatcgagaaaacaatctccaaagccaaaggcagccccga
gaaccacaggtgtacacctgccccatcccggtgagctgaccaagaaccaggtcagcctgacctgctgtgtaaaggcttct
atcccagcgacatcgctgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccggtggtgactcc
50 gacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgca
tgaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaatgatctagaaa

Amino acid sequence:

MPACRLGPLAAALLLSLLFGFTLVSGTGAMRTEDLPKAVVFLEPQWYRVLEKDS
 VTLKCQGA YSPEDNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSD
 5 PVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFH
 HNSDFYIPKATLKDSGSYFCRGLFGSKNVSSETVNITITQGLADQEPKSSDKTHTSP
 PSSAPELLGGSSVFLFPPKPKDTLMISRTPVTCVVVDVSHEDPEVKFNWYVDGVE
 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
 10 AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

102. a. CD16 ED low (native leader)

Nucleotide sequence:

aagcttgccgcatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgaggactgaagatctcccaaagg
 15 ctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggacagtgtagctctgaagtgccaggaggagcctactcccctgag
 gacaattccacacagtggtttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgctgccacagtcgacgac
 agtggagagtacaggtgccagacaaacctctccaccctcagtgaccgggtgcagctagaagtccatcgcggtggctgttgcctca
 ggcccctcggtgggtgttcaaggagggaagaccctattcacctgaggtgtcacagctggaagaacactgctctgcataaggtcacat
 attacagaatggcaaaggcagggaagtatttcatcataattctgacttctacattccaaaagccacactcaaagacagcggctcctac
 20 ttctgcagggggcttgtgggagtaaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggtttggctgatcaaaa

Amino acid sequence:

mwqlllptallllvsagmrtedlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassyfidaatvdds
 25 geyrcqtnlstlspvqlevhigwlllqaprwvfkeedpihlrchswkntalhkvtylqngkgrkyfhnnsdfyipkatlkds
 syfcrglvgsknvssetvnititqgladq

b. CD16 ED low (HE4 leader)

Nucleotide sequence

aagcttgccgcatgcctgcttgcgcctaggccgctagccgccgcccctcctcagcctgctgctgttcggcttcaccctagtct
 30 caggcaccgggtgcaatgcggactgaagatctcccaaaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaagg
 acagtgtagctctgaagtgccaggaggagcctactcccctgaggacaattccacacagtggtttcacaatgagagcctcatctcaagc
 caggcctcgagctacttcattgacgctgccacagtcgacgacagtgagagtagcaggtgccagacaaacctctccaccctcagtg
 acccggtgcagctagaagtccatcgcggtggctgttgcctccaggccccctcggtgggtgttcaaggagggaagaccctattcacctg
 aggtgtcacagctggaagaacactgctctgcataaggtcacatattacagaatggcaaaggcagggaagtatttcatcataattctg
 35 acttctacattccaaaagccacactcaaagacagcggctcctacttctgcagggggcttgtgggagtaaaaatgtgtcttcagaga
 ctgtgaacatcaccatcactcaaggtttggctgatcaaaa

Amino acid sequence

mpacrlgp laaallsl lfgftlvsgtgamrtedlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqass
 40 yfidaatvddsgeyrcqtnlstlspvqlevhigwlllqaprwvfkeedpihlrchswkntalhkvtylqngkgrkyfhnnsdf
 yipkatlkds gsyfcrglvgsknvssetvnititqgladq

103. a. CD16 ED high (native leader)

Nucleotide sequence:

gttgtaagcttgccgcatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgaggactgaagatctccc
 aaaggctgtggtgttcttgagcctcaatggtacaggggtgctcgagaaggacagtgtagctctgaagtgccaggaggagcctactcc
 cctgaggacaattccacacagtggtttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgctgccacagtc
 50 gacgacagtgagagtagcaggtgccagacaaacctctccaccctcagtgaccgggtgcagctagaagtccatcgcggtggctgt
 tgcctcaggccccctcggtgggtgttcaaggagggaagaccctattcacctgaggtgtcacagctggaagaacactgctctgcataag

gtcacatatttacagaatggcaaaggcaggaaagtattttcatcataattctgacttctacattccaaaagccacactcaaagacagcg
gctcctactctgcagggggccttttgggagtaaaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggtttggctgatcaa
a

5 Amino acid sequence:

mwqlllptallllvsagmrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassyfidaatvdds
geyrcqtnlstlsdpvqlvhighwlllqaprwvfkeedpihlrchswkntalhkvtlylqngkgrkyfhnsdfyipkatlkds
syfcrglfsgsknvssetvnititqladq

10 **b. CD16 ED high (HE4 leader)**

Nucleotide sequence:

aagcttgccgccatgctgtgtgcctaggccccgtagccgccgcccctcctcctcagcctgctgctgttcggcttcaccctagtct
caggcaccggtgcaatgcggactgaagatctcccaaaggctgtggtgttcctggagcctcaatggtacaggggtgctcgagaagg
acagtgtgactctgaagtgccaggagcctactcccctgaggacaattccacacagtgtttcacaatgagagcctcatctcaagc
15 caggcctcgagctacttcattgacgctgccacagtcgacgacagtggagagtacaggtgccagacaaacctctccaccctcagt
accgggtgcagctagaagtccatcgcgctggctgttgcctcaggccccctcggtgggtgttcaaggaggaagaccctattcacctg
aggtgtcacagctggaagaacactgctctgcataagggtcacatatttacagaatggcaaaggcaggaaagtattttcatcataattctg
acttctacattccaaaagccacactcaaagacagcgctcctacttctgcagggggccttttgggagtaaaaatgtgtcttcagagac
tgtgaacatcaccatcactcaaggtttggctgatcaaa

20

Amino acid sequence:

MPACRLGPLAAALLLSLLFGFTLVSGTGAMRTEDLPKAVVFLEPQWYRVLEKDS
VTLKCQGAYSPEDNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLS
PVQLEVHIGWLLQAPRWVFKEDPIHLRCHSWKNTALHKVTLYLQNGKGRKYFH
25 HNSDFYIPKATLKDSGSYFCRGLFGSKNVSSETVNITITQGLADQ

104. 2e12 scFv (SSS-S)H P238S CH2 WCH3-hCD80TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccc
30 aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaagtggtgaatattatgtcacaaagt
taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggtccctgcc
agggttagtgagcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttgagcgttcggtggaggcaccaagctggaaatcaaacgggggtggcgtggctcggcgagggtggg
tcgggtggcgggcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
35 accgtctcaggggttctcattaaccgggtatggtgtaaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atgggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtcttct
aaaaatgaacagcttgcaaaactgatgacacagccagatactactgtgccagagatgggtatagtaactttcattactatgtatggact
actgggggtcaaggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacatccccaccgtcccca
gcacctgaactcctggggggatcgtcagcttctccttccccccaaaacccaaggacaccctcatgatctccggaccctgaggt
40 cacatgcgtggtggtgagcgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgc
caagacaaagccgcgggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctga
atggcaaggagtagaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacctctcaaagccaaagggcag
ccccgagaaccacaggtgtacacctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaa
ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgt
45 ggactccgacggctccttctctctacagaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgtcc
gtgatgcagaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaagcggatccttcgaacctgctccc
atcctgggccattaccttaatctcagtaaatggaattttgtgatatgctgcctgacctactgctttgccccaaagtgcagagagagaa
ggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgat

50 Amino acid sequence:

MDFQVQIFSLLISASVMSRQVLDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
 CQQSRKVPWTFGGGKLEIKRGGGGSGGGGSGGGGSGVQLKESGPGLVAPSQSL
 ITCTVSGFSLTGYGVNWVRQPPGKLEWLGMIWGDGSTDYNSALKSRLSITKDNS
 5 .KSQVFLKMNSLQTDRTARYYCARDGYSNFHYVMDYWGQGTSTVTVSSDLEPKS
 SDKTHTSPSPAPPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTTPVLDSDGSFFLYSLKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL
 10 SLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

105. 10A8 scFv (SSS-S)H P238SCH2 WCH3-hCD80TM/CT

Nucleotide sequence:

aagcttatggatttcaagtcgagattttcagcttctgctaatacagtcagtcagtcataatgtccagaggagtcgacatccagatgaca
 15 cagctctccatcctcactgtctgcatctctgggaggcaaaagtcaccatcacttgcaaggcaagcaagacattaagaagtatataggtt
 ggtaccaacacaagcctggaaaagggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaagggtcagtg
 gaagtggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactattattgtcaacagtatgataat
 ctccattgacgttcggctcggggacaaaagttggaaataaacggggtggcgggtggctcgggcgggtggtgggtcgggtggcggc
 20 ggaatctgatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtcctctgtctctcacctgctctgtcactggctactc
 catcaccagtggtttctactggaactggatccgacagtttccgggaaacaaactggaatggatgggccacataagccacgacggta
 ggaataactacaacctatctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttctgaagttgagttctgtga
 ctactgaggacacagctacatatttctgtgaagacactacggtagtagcggagctatggactactggggtcaaggaacctcagtc
 accgtctcctctgatctggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctgaactcctggggggatc
 25 gtcagttcttcttcccccaaaacccaaggacacctcatgatctccggaccttgagggtcacatgctggtggtggacgtga
 gccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggagga
 gcagtacaacagcacgtaccgtgtggtcagcgtctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgcga
 ggtctccaacaaagccctccagccccatcgagaaaaccatctccaaaggccaaaggccagccccgagaaccacaggtgtaca
 30 ccctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgctgtaaaaggcttctatccagcgacatcgc
 cgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctggactccgacgctccttctctct
 ctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaac
 cactacacgcagaagacctctccctgtctccgggtaaagcggatccttgcgaacctgctccatcctgggccattacctaatactca
 gtaaatggaattttgtgatgtgctgacctactgcttgcaccaagatgcagagagagaaggagggaatgagagattgagaagg
 gaaagtgtacgcctgtataaatcgat

35 Amino acid sequence:

MDFQVQIFSLLISASVMSRQVLDIVLTQSPSSLSASLGKVTITCKASQDIKKYIG
 WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLIRNLEPEDIATYYCQQY
 DNLPLTFGSGTKLEIKRGGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSV
 TGYSITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFL
 40 KLSSVTTEDTATYFCARHYGSSGAMDYWGQGTSTVTVSSDLEPKSSDKTHTSPSP
 APELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
 GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
 VLDSDGSFFLYSLKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKADPS
 45 NLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

106. 2H7 scFv VHL11S (SSS-P)H P238SCH2CH3-hCD80TM/CT

Nucleotide sequence:

50 aagcttgcggccatggatttcaagtcgagattttcagcttctgctaatacagtcagtcagtcataatgccagaggacaaattgttctct
 cccagctctcagcaatcctgtctgcatctccaggggagaagggtcacaatgacttgcaaggccagctcaagtgtaagtacatgcact

ggtaccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagt
 gcagtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgaggatt
 taaccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtgctcggcggtggtggatctggaggaggtg
 ggagctctcaggcttattacagcagctctggggtgagtcggtgagcgctggggcctcagtgaagatgctctgaaggcttctggc
 5 tacacattaccagttacaatatgactgggtaagcagacacctagacagggcctggaatggattggagctattatccaggaaat
 ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
 cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
 agggaccacggtcaccgtctctctgatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaact
 cctggggggatcgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccggaccttgaggtcacatgcgtgg
 10 tgggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaagacaaagc
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 acaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatccc
 agcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
 15 gctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtgatgcagag
 gctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaagcggatccttcgaacctgctccatcctgggccatt
 acctaatctcagtaaatggaattttgtgatgtgctgacctactgcttggcccaagatgcagagagagaaggaggaatgaga
 gattgagaagggaagtgtacgccctgtataaatgat

20 Amino acid sequence:

MDFQVQIFSLLISASVILARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
 QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
 FNPPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAESVRPGASVKMSCK
 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
 25 TAYMQLSSLTSEDSAVYFCARVVYYNSYWFYFDVWGTGTTVTVSSDLEPKSSDK
 THTSPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSGSSFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS
 30 PGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

107. G19-4 scFv (SSS-P)H P238SCH2 WCH3-hCD80TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagatttcagcttctctgtaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
 35 cagactacatctccctgtctgcctctctgggagacagagtcaccatcagttgcagggaagtcaggacattcgcaattattaaact
 ggtatcagcagaaaccagatggaactgttaactcctgatctactacacatcaagattacactcaggagtcctcatcaaggttcagt
 gcagtgggtctggaacagattattctctcaccattgccaacctgcaaccagaagatattgccacttactttgccaacagggtataac
 gcttccgtggacgttcggtggaggcaccaactggtaacaaacgggagctcgggtggcggtggtcggcggtggtgggtcgg
 gtggcggggatctatcgatgaggtccagctgcaacagctctggacctgaactggtgaagcctggagcttcaatgtcctgcaaggc
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 cctgaactcctggggggtatcgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccggaccttgaggtcac
 45 atgcgtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaa
 gacaaagccgcgggaggagcagtaaacagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatg
 gcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccc
 cgagaaccacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaagg
 ctctatcccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctgg
 50 actccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtg
 atgcatgaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaagcggatccttcgaacctgctcccatc

Amino acid sequence:

108. **2e12 scFv IgA WH WCH2 T4 CH3-hCD80TM/CT**

20 aagccttatggatttcaagtgacagatttccagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc
aatctccagcttcttggctgtgtctctaggtcagagagccaccatctctgcagagccagtgaaagtgtgaatattatgtcacaagtt
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actgggggtcaaggaacctcagtcaccgtctcctcagatcagccagttccctcaactccacctacccatctccctcaactccaccta
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35 ccagggcaccaccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcat
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gttgcacatggcggaggtggacgcggatccttgaacaacctgctccatcctgggccattaccttaatctcagtaaatggaattttgt
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45
50

MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYP
CQQRKVPWTFGGGKLEIKRGGGSGGGGSGGGGSGVQLKESGPGLVAPSQSL
ITCTVSGFSLTG YGVNWVRQPPGKLEWLGMTWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARGYSNFHYVMDYWGQGTSTVTVSSDQPVPS
TPPTSPSTPPTSPSCCHPRLSLHRPALEDLLGSEAILTCTLTGLRDASGVTFTWTP
SSGKSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFCTAAYPESKTPLTATLS
KSGNTRPEVHLLPPPSEELALNELVTLTCLARGFSPKDLVRWLQGSQELPREKY
LTWASRQEPSQGTTFVAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTOKTIDR

LAGKPTHVNVSVVMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRER
RRNERLRRESVRPV

109. 2e12 scFV (SSS-P)H P238S CH2 WCH3-mFADD-TM/CT

5 Nucleotide sequence:

aagcttatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcacc
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aggttttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
10 aaagtaggaaggttccttggacgttcggtggaggcaccgaagtggaaatcaaacgggggtggcgggtggctcgggcggagggtggg
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tggcaaggagtacaagtgaaggtctccaacaagccctccagcccccatcgagaaaaccatctccaagccaaagggcagc
20 cccgagaaccacaggtgtacaccctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgctggtcaag
gcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccggtgctg
gactccgacggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaactcttctcatgctccgt
gatgcatgaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaagcggtatcctgaacatggacctat
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25 aaacgaaagctggagcgcgtgcagagtggcctggacctgttcacggtgctgctggagcagaacgacctggagcgcgggcaca
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gagctgaaaagctggaagaatgctgagaagaagaagcctcggtggccggactggtcaaggcgctgcggacctgcaggctga
30 atctggtggtgacctggtggaagaagcccaggaatctgtgagcaagagtgagaatatgtcccagactaagggaattcaactgtg
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Amino acid sequence:

MDFQVQIFSLLISASVMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
35 LMQWYQQKPGQPPLKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSL
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQGSTVTVSSDLEPKS
SDKTHTSPPSPAPPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
40 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSL
SLSPGKADPSNMDPFLVLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDL
FTVLLEQNDLERGHTGLLRELLASLRHDLQLRLDDFEAGTATAAPPGEADLQVA
45 FDIVCDNVGRDWKRLARELVSEAKMDGIEEKYPRSLSERVRESLKVWKNAEKK
NASVAGLVKALRTCRNLVADLVEEAQESVSKSENMSPLRDSTVSSSETP

110. 2e12 scFv (SSS-P)H WCH2WCH3-mFADD-TM/CT

Nucleotide sequence:

50 aagcttatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcacc
aatctccagcttcttggctgtgtctctaggtcagagagccaccatctctgcagagccagtgaagtgtgaatattatgtcacaagtt

taatgcagtgggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctgggggtccctgcc
 aggttttagtggcagtgggtctgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtatttctgtcagc
 aaagtaggaagggttccttgacgttcggtggaggcaccaagctggaaatcaaacgggggtggcgggtggctcgggcggagggtggg
 tcgggtggcggcggatctcaggtgcagctgaaggagtcaggacctggcctgggtggcggcctcacagagcctgtccatcacatgc
 5 accgtctcagggttctcattaaccggctatggtgtaaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
 atgggggtgatggaagcacagactataattcagctctcaaataccagactgagcatcaccaaggacaactccaagagccaagtcttctt
 aaaaatgaacagtctgcaaactgatgacacagccagataactactgtgccagagatgggtatagtaactttcattactatgttatggact
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 caagacaaagccgcgggaggagcagtacaacagcacgtacctgtggtcagcgtcctcaccgtcctgcaccaggactggctga
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 acaccgggctgctgcgcgagttgctggcctcgtgcgccgacacgatctactgcagcgcctggacgacttcgaggcggggacg
 20 gcgaccgctgcgccccgggggagggcagatctcaggtggcatttgacattgtgtgtgacaatgtgggggagagactggaaaaga
 ctggcccgcgagctgaaggtgtctgaggccaagatggatgggattgaggagaagtacccccgaagtctgagtgcgggtaag
 ggagagtctgaaagtctggaagaatgctgagaagaagaacgcctcgggtggccggactgggtcaaggcgtgcggacctgcaggc
 tgaatctgggtggctgacctgggtggaagaagcccaggaatctgtgagcaagagtgagaatatgtccccagtactaagggttcaact
 gtgtcttctcagaaacacctgactcgagatcgat

Amino acid sequence:

MDFQVQIFSLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
 CQQSRKVPWTFGGGTKLEIKRGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSL
 30 ITCTVSGFSLTGYGVNWVRQPPGKLEWLGMWGDGSTDYNSALKSRLSITKDNS
 KSQVFLKMNSLQTDRTARYYCARDGYSNFHYVMDYWGQGSVTVSDDLPEPKS
 SDKTHTSPSPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 35 PENNYKTTTPVLDSGDFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
 SLSPGKADPSNMDPFLVLLHSLSGSLSGNDLMELKFLCRERVSKRKLERSVQGLDL
 FTVLLEQNDLERGHTGLLRELLASLRHDLQLRLDDFEAGTATAAPPGEADLQVA
 FDIVCDNVGRDWKRLARELKVSEAKMDGIEEKYPRSLSERVRESLKVKNAEKK
 NASVAGLVKALRTCLNLVADLVEEAQESVSKSENMSPLRDSTVSSSETP

111. mcasp3-TM/CT

Nucleotide sequence:

Ggatcctcgaacatggagaacaacaaacctcagtgattcaaatccattaataatttgaagtaaagaccatacatgggagcaa
 gtcagtggactctgggatctatctggacagtagttacaaaatggattatcctgaaatgggcatatgcataataattaataagaactt
 45 ccataagagcactggaatgtcatctcgtctgtacggatgtggacgcagccaacctcagagagacattcatgggcctgaaatacc
 aagtcaggaataaaaatgatcttactcgtgaagacatttgaattaatggatagtttctaaggaagatcatagcaaaaggagcag
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 50 acctggttactattcctggagaaattcaaaggacgggtcgtggttcatccagtccttgcagcatgctgaagctgtacgcgcacaa

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Amino acid sequence:

5 DPSNMENNKTSDSKSINNFEVKTIHGSKSVDSGIYLDSSYKMDYPEMGICIIINNK
NFHKSTGMSSRSGTDVDAANLRETFMGLKYQVRNKNDLTREDILELMDSVSKED
HSKRSSFVFCVILSHGDEGVITYGTNGPVELKCLTSFFRGDYCRSLTGKPKLFIQACR
GTELDGCIETDSGTDEEMACQKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSL
10 CSMLKLYAHKLEFMHILTRVNRKVATEFESFSLDSTFHAKKQIPCIVSMLTKEL YF
YH

112. 2e12 scFv (SSS-P)H WCH2WCH3-mcasp3-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccc
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40 actcgcgttaacaggaaggtggcaacggaattcagtccttctccctggactccactttccacgcaaaagaacagatcccgtgtatt
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Amino acid sequence:

45 MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAM YF
CQQSRKVPWTFGGGKLEIKRGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSL
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTD TARYYCARGYSNFHYVMDYWGQGSTVTVSSDLEPKS
SDKTHTSPSPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
50 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ

PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSL
 SLSPGKADPSNMENNKTSVDSKSNINFEVKTIHGSKSVDSGIYLDSSYKMDYPEMG
 ICIINNKNFHKSTGMSSRSGTDVDAANLRETFMGLKYQVRNKNLDTREDILELMD
 SVSKEDHSKRSSFVCVILSHGDEGVITYGTNGPVELKKLTSFFRGDYCRSLTGKPKLF
 5 IIQACRGTELDGCIETDSGTDEEMACQKIPVEADFLYAYSTAPGYYSWRNSKDGS
 WFIQSLCSMLKLYAHKLEFMHILTRVNRKVATEFESFSLDSTFHAKKQIPCTVSMLT
 KELYFYH

10 113. 2e12 scFv (SSS-P)H P238SCH2WCH3-mcasp3-TM/CT

Nucleotide sequence:

aagcttatggatttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccc
 aatctccagcttcttggctgtgtcttaggtcagagagccaccatctctgcagagccagtgaaagtgtgaatattatgcacaagtt
 taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctgtgcatcaacgtagaatctggggtccctgcc
 15 aggttttagtgagcagtggtgtgggacagacttcagcctcaacatccatcctgtggaggaggtgatattgcaatgtatttctgtcagc
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 35 cggagctgactggaaagccgaaactcttcattcagcctgccggggtacggagctggactgtggcattgagacagacagtg
 ggactgatgaggagatggcttccagaagataccggtggaggtgacttctgtatgcttactctacagcacctggttactatcctg
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 ctcactcgcgttaacaggaaggtggcaacggaattcagtccttctccttgactccacttccacgcaagaacagatcccgtgt
 attgtgtccatgtcacgaaagaactgtactttatcactagctcgagatcgatga

Amino acid sequence:

MDFQVQIFSLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPLKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYP
 CQQSRKVPWTFGGGKLEIKRGGGGSGGGGSGGGGSGVQLKESGPGLVAPSQSL
 45 ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
 KSQVFLKMNSLQTDRTARYYCARGYSNFHYVMDYWGQGTSTVTVSSDLEPKS
 SDKTHTSPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 50 PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSL
 SLSPGKADPSNSNMENNKTSVDSKSNINFEVKTIHGSKSVDSGIYLDSSYKMDYPE

MGICIIINNKNFHKSTGMSSRSGTDVDAANLRETFMGLKYQVRNKNDLTREDILEL
MDSVSKEDHSKRSSFVCVILSHGDEGVYGTNGPVELKKLTSFFRGDYCRSLTGKP
KLFIIQACRGTELDGCIETDSGTDEEMACQKIPVEADFLYAYSTAPGYYSWRNSKD
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5 LTKELYFYH

114. mcasp8-TM/CT

Nucleotide sequence:

ttcgaacatggatttcagagtgctttatgctattgctgaagaactgggcagtgagacctggctgccctcaagttcctgtgcttga
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25 agggaaagcagatgccacagcccaccttcacactacggaagaagctcttctccctccctaatgactcgagatcgatt

Amino acid sequence:

SNMDFQSCLY AIAEELGSEDLAALKFLCLDYIPHKKQETIEDAQKLFLRLREKGMLE
30 EGNLSFLKELLFHISRWDLLVNFLDCNREEMVREL RDP DNAQISPYRVMFLKLSE
EVSELELR SFKFLNNEIPKCKLEDDLSLLEIFVEMEKRTMLAENNLETLSICDQV
NKSLLGKIEDYERSSTERRMSLEGREELPPSVLDEMSLKMAELCDSPREQDSESRTS
DKVYQMKNKPRGYCLINNHDFSKAREDTQLRKMMDRKGTDCDKEALSKTFKEL
HFEIVSYDDCTANEIHEILEGYQSADHKNKDCFICILSHGDKGVVYGTGKEASIY
35 DLTSYFTGSKCPSLSGKPKIFFIIQACQGSNFQKGV PDEAGFEQQNH TLEV DSSSHKN
YIPDEADFL LGMATVKN CVSYRDPVNGTWYIQSLCQSLRERCPQGDDILSIL TGVN
YDVS NKDDRRNK GKQMPQPTFTLRKKLFFPP

40 115. 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp8-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc
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45 taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctgtgcatccaacgtagaatctggggtccctgcc
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 10 agaagcaggagaccatcgaggtatcccagaagctatttctgaggtgcgggaaaaggggatgttggaggaaggcaatctgtcttt
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 15 attatgaagatcaagcacagagagaagaatgagcctgaagggaagggaaggttgcaccttcagtttggatgagatgagcctc
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 20 gtgacaagggtgtctgtatggaacggatgggaaggaggcctccatctatgacctgacatcttacttactggttcaaagtgccttc
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Amino acid sequence:

MDFVQVQIFSLLISASVMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYP
 30 CQQSRKVPWTFGGGTKLEIKRGGGSGGGGSGGGGSGVQLKESGPGLVAPSQSL
 ITCTVSGFSLTGYGVNWVRQPPGKLEWLGMIWGDGSTDYNSALKSRLSITKDNS
 KSQVFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQTSVTVSSDLEPKS
 SDKTHTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 35 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSL
 SLSPGKADPSNMDFAQSCLYALAEELGSEDLAALKFLCLDYIPHKKQETIEDAQKLFL
 RLREKGMLEGNLSFLKELLFHISRWDLLVNFLDCNREEMVRELDPDNAQISPYR
 VMLFKLSEEVSELELRSFKFLNNEIPKCKLEDDLSLLEIFVEMEKRTMLAENNLET
 40 LKSICDQVNKSLGKIEDYERSSTERRMSLEGREELPPSVLDMSLKMALCDSR
 EQDSESRSDKVYQMKNKPRGYCLINNHDFSKAREDTQLRKMMDRKGTDCDKE
 ALSKTFKELHFEIVSYDDCTANEIHEILEGYQSADHKNKDCFICILSHGDKGVVYG
 TDGKEASIIDLTSYFTGSKCPSLSGKPKIFFIQACQGSNFQKGVPEAGFEQQNHTL
 EVDSSSHKNYIPDEADFLGMATVKNCSVSRDPVNGTWYIQSLCQSLRERCPQGD
 45 DILSILTVNYDVSNDKDDRRNKGKQMPQPTFTLRKKLFFPP

116. 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-mcasp8-TM/CT

Nucleotide sequence:

aagcttatggatttcaagtgcagatttccagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccc
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 aaggagatgacattcttagcatcctgactggcgtgaactatgacgtgagcaataaagacgacagggaagaacagggaagcaga
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Amino acid sequence:

35 MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYP
 CQQRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSL
 ITCTVSGFSLTGYGVNWVRQPPGKLEWLGMWGDGSTDYNSALKSRLSITKDNS
 40 KSQVFLKMNSLQDDTARYYCARDGYSNFHYVMDYWGQGTSTVTVSSDLEPKS
 SDKTHTSPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTTPVLDSGSSFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSL
 45 SLSPGKADPSNMDFQSLYAIAEELGSEDLAALKFLCLDYIPHKKQETIEDAQKLFL
 RLREKGMLEEGNLSFLKELLFHISRWDLNVNFLDCNREEMVRELDPDNAQISPYR
 VMLFKLSEEVSELELRSFKFLNNEIPKCKLEDDLSLLEIFVEMEKRMTLAENNLET
 LKSICDQVNKSLLGKIEDYERSSTERRMSLEGREELPPSVLDEMSLKMAELCDSPR
 EQDSESRTSDKVYQMKNKPRGYCLINNHDFSKAREDTQLRKMMDRKGTDCDKE
 50 ALSKTFKELHFEIVSYDDCTANEIHEILEGYQSADHKNKDCFICILSHGDKGVVYG
 TDGKEASIYDLTSYFTGSKCPSLSGPKIFFIQACQGSNFQKGVPEAGFEQQNHTL

EVDSSSHKNYPDEADFLGMATVKNVSYRDPVNGTWYIQLCQSLRERCPQGD
DILSILTGVNYDVS NKDDRRNK GKQMPQPTFTLRKKLFFPP

5 **117. hcaspl-TM/CT**

Nucleotide sequence:

Ggacccctcgaacatggagaacactgaaaactcagtggaattcaaaatccattaaaaatttgaaccaaagatcatacatggaagcg
aatcaatggactctggaatatccctggacaacagttataaaatggattatcctgagatgggtttatgtataataataataagaatttt
cataaaagcactggaatgacatctcggctcgtgtacagatgtcgtatgcagcaaactcagggaacattcagaaacttgaatatga
10 agtcaggaataaaaaatgatcttacacgtgaagaaattgtgaattgatcgtgatgtttctaaagaagatcacagcaaaaggagcag
ttttgtttgtgcttctgagccatgggtgaagaaggaaataattttggaacaaatggacctgttgacctgaaaaaataacaaacttttc
agaggggatcgtttagaagttaactgaaaacccaaacttttcattattcaggcctgccgtggtacagaactggactgtggcattg
agacagacagtggtgttgatgatgacatggcgtgtcataaaataccagtgaggccgacttctgtatgcatactccacagcacctg
gttattattcttggcgaaattcaaaggatggctcctgggtcatccagtcgcttctgtccatgctgaaacagtatgccgacaagcttgaat
15 ttatgcacattcttaccgggtaaccgaaagggtggcaacagaatttgagtcctttccttgacgctactttcatgcaaaagaacaga
ttccatgtattgttccatgctcacaagaactctattttatcactaactcagatcgata

Amino acid sequence:

DPSNMENTENSVDKSIKNLEPKIIHGSESMDSGISLDNSYKMDYPEMGLCIINNK
20 NFHKSTGMTSRSGTDVDAANLRETFRNLYEVRNKNLDTREEIVELMRDVSKED
HSKRSSFVVCVLLSHGEEGIIFGTNGPVDLKKITNFFRGDRCSRSLTGKPKLFIIQACRG
TELDGCIETDSGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLC
AMLKQYADKLEFMHILTRVNRKVATEFESFSFDATFHAKKQIPICIVSMLTKELYFY
H

25 **118. 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcaspl-TM/CT**

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc
aatctccagcttcttggctgtgtcttaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaattatgtcacaagtt
30 taatgcagtggtaccaacagaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggtccctgcc
agggttagtgagcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtattctgtcagc
aaagtaggaaggttccctggacgttcggtggaggacccaagctggaaatcaaacgggggtggcgggtggctcggcgagggtggg
tcgggtggcggcgatctcaggtgcagctgaaggagtcaggacctggcctgggtggcgccttcacagagcctgtccatcacatgc
accgtctcaggggttctcattaaccggctatgggtgtaactgggtcggcagcctccaggaaagggtctggagtggtgggaatgat
35 atgggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttctt
aaaaatgaacagctgcgaactgatgacacagccagatactactgtccagagatggttatagtaactttcattactatgttatggact
actgggggtcaaggaacctcagtcaccgtctcctcagatctggagcccaaatctctgacaaaactcacacatccccaccgtcccca
gcacctgaactcctgggtggaccgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccgaccctgagggt
cacatgcgtggtggtggacgtgagccacgaagacctgagggtcaagttaactggtacgtggacggcgtggagggtgcataatgc
40 caagacaaagccgcgggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctga
atggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcgagaaaccatctccaaagccaaagggcag
ccccgagaaccacaggtgtacacctgccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgctgtgctaaa
ggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccggtgct
ggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcc
45 gtgatgatgaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaagcggtatcttgaacatggaga
aactgaaaactcagtggaattcaaaatccattaaaaatttgaaccaaagatcatacatggaagcgaatcaatggactctggaatatc
cctggacaacagttataaaatggattatcctgagatgggtttatgtataataataataagaattttcataaaagcactggaatgaca
tctcggctcgtgtacagatgtcgtatgcagcaaactcagggaacattcagaaacttgaaatatgaagtcaggaataaaaaatgatctta
cacgtgaagaaattgtggaattgatcgtgatgtttctaaagaagatcacagcaaaaggagcagttttgtgtgtgcttctgagccat
50 ggtgaagaaggaaataattttggaacaaatggacctgtgacctgaaaaaataacaaacttttcagaggggatcgtttagaagtc
taactggaaaacccaaacttttcattattcaggcctgccgtggtacagaactggactgtggcattgagacagacagtggtgttgatga

tgacatggcgtgtcataaaataaccagtgaggccgacttctgtatgcatactccacagcacctggttattattcttggcgaaattcaa
 aggatggctcctggttcatccagtcgcttgtgccatgctgaaacagtatgccgacaagctgaattatgcacattcttaccggggtta
 accgaaagggtggcaacagaatttgagtcctttcctttgacgctactttcatgcaagaacagattccatgtattgttccatgctcac
 aaaagaactctattttatcactaactcgagatcgata

5

Amino acid sequence:

MDFQVQIFSFLISASVMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
 CQQSRKVPWTFGGGKLEIKRGGGSGGGGSGGGGSGVQLKESGPGLVAPSQSLS
 10 ITCTVSGFSLTGYGVNWVRQPPGKLEWLGMWGDGSTDYNSALKSRLSITKDNS
 KSQVFLKMNSLQTDRTARYYCARGYSNFHYVMDYWGQGTSTVTVSSDLEPKS
 SDKTHTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 15 PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL
 SLSPGKADPSNMENTENSVDKSIKNLEPKIHGSESMDSGISLDNSYKMDYPEMG
 LCIINNKNFHKSTGMTSRSGTDVDAANLRETFRNLKYEVRNKNLDTREEIVELMR
 DVSKEDHSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNFRGDRCSLTGKPKLFI
 IQACRGTELDGCIETDSGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGS
 20 WFIQSLCAMLKQYADKLEFMHILTRVNRKVATEFESFSFDATFHAKKQIPCIVSML
 TKELYFYH

123. 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp3-TM/CT

Nucleotide sequence:

25 aagcttatggatttcaagtgcagattcagcttctgtaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcacc
 aatctccagcttcttggctgtgtcttaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtcacaagtt
 taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
 aggttttagtgagcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
 aaagtaggaaggttccttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcggtggctcgggcggaggtggg
 30 tcgggtggcgccggatctcaggtgcagctgaaggagtcaggacctggcctgtggcgccctcacagagcctgtccatcacatgc
 accgtctcagggttctcattaaccggctatggtgtaactgggttcgccagcctccaggaaagggtctggagtggtgggaatgat
 atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagaccaagtttctt
 aaaaatgaacagctctgcaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggact
 actgggtgcaaggaacctcagtcaccgtctcctcagatctggagcccaatcttctgacaaaactcacacatccccaccgtcccca
 35 gcacctgaactcctgggtggatcgtcagttcttcttcccccaaaaccaaggacacctcatgatctccggacccctgaggtc
 acatgcgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcc
 aagacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaa
 tggcaaggagtagaagtgcaaggtctcaacaaagccctccagccccatcgagaaaaccatctcaagccaaagggcagc
 cccgagaaccacaggtgtacacctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctggtcaag
 40 gcttctatcccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctg
 gactccgacggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaaccttctcatgctccgt
 gatgcatgaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaacggatccttgaacatggagaac
 actgaaaactcagtgattcaaaatccattaaaaatttgaaccaaagatcataatggaagcgaatgaatggactctggaatatccc
 tggacaacagttataaaatggattatcctgagatgggtttatgtataataataataagaattttcataaaagcactggaatgacatc
 45 tcggtctggtacagatgctgatgcagcaaacctcagggaaacattcagaaactgaaatgaagtcaggaataaaaatgatcttac
 acgtgaagaaattgtgaattgatcggtgatgttctaaagaagatcacgcaaaaggagcagtttgtttgtgcttctgagccatg
 gtgaagaaggaaataattttggaacaaatggacctgtgacctgaaaaaataacaaacttttcagaggggagcgtgtgtagaagct
 aactggaaaacccaaacttttcattattcaggcctgccgtggtacagaactggactgtggcattgagacagacagtggtgttgatgat
 gacatggcgtgtcataaaataaccagtgaggccgacttctgtatgcatactccacagcacctggttattattcttggcgaaattcaaa
 50 ggatggctcctggttcatccagtcgcttgtgccatgctgaaacagtagtccgacaagcttgaattatgcacattcttaccggggttaa

ccgaaaggtggcaacagaatttgagtccttttccttgacgctacttttcacgaaagaacagattccatgtattgtttccatgctcaca
aaagaactctattttatcactaactcgagatcgataa

Amino acid sequence:

5 MDFQVQIFSFLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMFY
CQQRKVPWTFGGGKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
10 KSQVFLKMNSLQTDRTARYYCARGYSNFHYVMDYWGQTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSL
15 SLSPGKADPSNMENTENSVDKSKIKNLEPKIIHGSESMDSGISLDNSYKMDYPEMG
LCIINNKNFHKSTGMTSRSGTDVDAANLRETFRNLYEVRNKNLDTREEIVELMR
DVSKEDEHRSKRSFVCLLSHGEEGIIFGTNGPVDLKKITNFFRGDRCSLTGKPKLFI
IQACRGTELDGIEGTDSDGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGS
WFIQSLCAMLKQYADKLEFMHILTRVNRKVATEFESFSFDATFHAKKQIPCIIVSML
TKELYFYH

20

124. hcas8-TM/CT

hCaspase8B:

Nucleotide sequence:

25 ggatccttcgaacatggacttcagcagaaatctttatgatattgggaacaactggacagtgaagatctggcctccctcaagttcctg
agcctggactacattccgaaaggaagcaagaacccatcaaggatgccttgatgtattccagagactccaggaagagaatgtt
ggaggaaagcaatctgtccttctgaaggagctgctctccgaattaatagactggattgctgattacctacctaactagaaag
gaggagatggaagggaacttcagacaccaggcagggctcaaatctgcctacagggtcatgctctatcagatttcagaagaagt
gagcagatcagaattgaggtctttaagttctttgcaagaggaaatctcaaatgcaactggatgatgacatgaacctgctggata
tttcatagagatggagaagaggggtcatcctgggagaaggaaagttggacatcctgaaaagagctgtgcccacaaatcaacaagag
30 cctgtgaagataatcaacgactatgaagaattcagcaaaagagagaagcagcagcctgaagggaagtcctgatgaatttcaaatg
gggaggagttgtgtgggtaatacgaatctcgactctcgaagagaacaggatagtgatcacagactttggacaaagttaccaa
atgaaaagcaaacctcgggatactgtctgatcatcaacaatcacaatttgcaaaagcacgggagaaagtgcacaaacttcacag
cattaggagcaggaatggaacacacttgatgcaggggctttgaccacgacctttgaagagcttcattttgagatcaagcccacg
atgactgcacagtagagcaaatctatgagattttgaaatctaccaactcatggaccacagtaacatggactgcttcatctgctgatac
35 ctctcccatggagacaagggtcatctatggcactgatggacaggaggccccatctatgagctgacatctcagttcactggtttg
aagtgccttccctgtggaacccaaagtgtttttattcaggctgtcaggggataactaccagaaaggtatacctgttgagac
tgattcagaggagcaaccctatttagaatgatttatcatcacctcaaacgagatatatcccgatgaggtgactttctgctgggg
atggccactgtgaataactgtgttctaccgaaacctgcagagggaacctggtacatccagtcactttgccagagcctgagaga
gcgatgtctcaggcgatgatattctaccatcctgactgaagtgaactatgaagtaagcaacaaggatgacaagaaaaacatgg
40 ggaacagatgcctcagcctactttcacactaagaaaaaactgtcttccctctgattgagcatgcatcgata

Amino acid sequence:

DPSNMDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQRLQEKR
MLEESNLSFLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRVMYQIS
45 EEVSRSELRSFKLLQBEISKCKLDDDMNLLDIFIEMEKRVILGEGKLDILKRVCAQI
NKSLLKIINDYEEFSKERSSSLEGGSPDEFSGEELCGVMTISDSPREQDSESQTLDKV
YQMKSKPRGYCLIINHNFAKAREKVPKLHSIRDRNGTHLDAGALTTTFFELHFEI
KPHDDCTVEQIYEILKIYQLMDHSNMDCFICILSHGDKGIYGTGQEAPIYELTS
QFTGLKCPSLAGKPKVFFIQACQGDNYQKGIPVETDSEEQPYLEMDLSSPQTRYIPD
50 EADFLGMATVNNCVSYRNPAGTWTYIQLSCQSLRERCPRGDDILTILTEVNYEVS
NKDDKKNMGKQMPQPTFTLRKKLVFSPD

hCaspase8C:

Nucleotide sequence:

5 ggatccttcgaacatggacttcagcagaaatctttatgatattggggaacaactggacagtgaagatctggcctccctcaagttcctg
 agcctggactacattccgcaaaggaagcaagaacccatcaaggatgccttgatgttattccagagactccaggaaaagagaatgtt
 ggaggaaagcaatctgtccttctgaaggagctgtcttccgaattaatagactggatttgctgattacctacctaacactagaaag
 gagagatggaaaggggaacttcagacaccaggcagggctcaaatctgcctacagggtcatgcttatcagatttcagaagaagt
 gagcagatcagaattgaggtcttttaagttcttttgcagagggaatctccaaatgcaaactggatgatgacatgaacctgctggata
 10 tttcatagagatggagaagaggggtcatcctgggagaaggaaagtggacatcctgaaaagagtctgtgcccaaatcaacaagag
 cctgctgaagataatcaacgactatgaagaattcagcaaaggggaggagttgtgtgggtaatgacaatctcggactctccaagag
 aacaggatagtgatcacagactttggacaaagtttaccaaatgaaaagcaaacctcggggatactgtctgatcatcaacaatcaca
 attttgcaaaagcacgggagaaagtgcccaaaacttcagcattagggacaggaatggaacacacttgatgcaggggctttgac
 cagcactttgaagagcttcattttgagatcaagccccacgatgactgcacagtagagcaaactctatgagatttgaaaatctacca
 ctcatggaccacagtaacatggactgcttcatctgtgtatcctctccatggagacaagggtcatctatggcactgatggacag
 15 gagggccccatctatgagctgacatctcagttcactggttgaaagtgccttcccttgctggaaaacccaaagtgtttttattcaggctt
 gtcaggggggataactaccagaaaggtatacctgttgagactgattcagaggagcaaccctatttagaaatggatttatcatcacctca
 aacgagatatacccggatgaggtgactttctgtggggatggccactgtgaataactgttttctaccgaaacctgcagaggg
 aacctggtacatccagtcactttgccagagcctgagagagcgaatgctctcaggcgatgatattctcaccatcctgactgaagtga
 ctatgaagtaagcaacaaggatgacaagaaaaacatggggaaacagatgcctcagcctactttcacactaagaaaaaaactgtct
 20 tcccttctgattgagcatgcatcgata

Amino acid sequence:

DPSNMDFSRNLIDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQRLQEKR
 MLEESNLSFLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRVMLEYQIS
 25 EEVSRSELRSFKFLQEEISKCKLDDDMNLLDIFIEMEKRVILGEGKLDILKRVCAQI
 NKSLLKIINDYEEFSKGEELCGVMTISDSPREQDSESQTLDKVYQMKSKPRGYCLII
 NNHNFARKAREKVPKLHSIRDRNGTHLDAGALTTTFFELHFEIKPHDDCTVEQIYEIL
 KIYQLMDHSNMDCFICILSHGDKGIYGTGQEAPIYELTSQFTGLKCPSLAGKPK
 VFFIQACQGDNYQKGIPVETDSEEQPYLEMDLSSPQTRYIPDEADFLGMATVNNC
 30 VSYRNP AEGTWYIQSLCQSLRERCPRGDDILTILTEVNYEVS NKDDKKNMGMKQMP
 QPTFTLRKKLVFSPD

125. 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcasp8-TM/CT

Nucleotide sequence:

35 aagcttatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgtcaccc
 aatctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaagtgttgatattatgtcacaaagt
 taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctgggtcctgcc
 aggttagtggtcagtggtgtgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
 aaagtaggaaggttccttgacgttcggtggagggaccaaagctggaaatcaaacgggggtggcggtggtcggcgagggtggg
 40 tcgggtggcggggatctcaggtgcagctgaaggagtcaggacctggcctgggtggcgccctcacagagcctgtccatcacatgc
 accgtctcagggttctcattaaccggctatggtgtaaactgggtcggcagcctccaggaaagggtctggagtggctgggaatgat
 atgggggtgatggaagcacagactataattcagctcclaaatccagactgagcatcaccaaggacaactccaagagccaagtttctt
 aaaaatgaacagcttgcaactgatgacacagccagatactactgtgccagagatggttagtaactttcattactatgttatggact
 actgggggtcaaggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtcccca
 45 gcacctgaactcctgggtggaccgtcagttctcttcccccaaaaacccaaggacacctcatgatctcccggaccctgaggt
 cacatgcgtggtgggtgacgtgagccacgaagacctgaggtcaagttaactggtagctggagcggcgtggaggtgcataatgc
 caagacaaagccgcgggaggagcagtaaacagcacgtaccgtgtgtgcagcgtcctaccgtcctgcaccaggactggctga
 atggcaaggagtagaagtgaaggtctccaacaaagccctccagccccatcgagaaaacctctccaaagccaaagggcag
 ccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaa
 50 ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccctgct
 ggactccgacggctccttctcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctcc

gtgatgcatgaggctctgcacaaccactacacgcagaagagccctcctgtctccgggtaaagcggatccttgaacatggactt
 cagcagaaatctttatgatattggggaacaactggacagtgaagatctggcctccctcaagttcctgagcctggactacattccgca
 aaggaagcaagaacctcatcaaggatgccttgatgttattccagagactccaggaaaagagaatgttgaggaaagcaatctgtcct
 tcctgaaggagctgctcttcgaattaatagactggaattgctgattacctacctaataactagaaaggaggagatggaaaggggaaac
 5 ttcagacaccaggcagggtctaaattctgcctacagggtcatgctctatcagatttcagaagaagtgagcagatcagaattgaggt
 ctttaagtttctttgcaagaggaaatctccaaatgcaaactggatgatgacatgaacctgctggatatttcatagagatggagaaga
 gggatcatcctgggagaaggaaagtggacatcctgaaaagagctgtgcccacaaatcaacaagagcctgctgaagataatcaacga
 ctatgaagaattcagcaaagagagaagcagcagccttgaaggagctctgatgaattttcaaatggggaggagttgtgtggggttaa
 10 tgacaatctcggactctcaagagaacaggatagtgaatcacagactttggacaaagttaccacaaatgaaaagcaaacctcgggga
 tactgtctgatcatcaacaatcacaattttgcaaaagcacgggagaaagtgcacaaacttcacagcattagggacaggaatggaac
 acacttggatgcaggggctttgaccacgacctttgaagagcttcattttgagatcaagccccacgatgactgcacagtagagcaaat
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 acccaaagtgtttttattcaggcttgcagggggataactaccagaaaggatatacctgttgagactgattcagaggagcaacctatt
 15 tagaaatggatttatcatcacctcaaacgagatatatccggatgaggctgactttctgctggggatggccactgtgaataactgtgtt
 tcctaccgaaacctgcagagggaacctgtgtacatccagtcactttgccagagcctgagagagcgtgtcctcgaggcgatgatata
 tctcaccatcctgactgaagtgaactatgaagtaagcaacaaggatgacaagaaaaacatggggaaacagatgcctcagcctactt
 tcacactaagaaaaaactgtcttcccttctgattgagcatgcatcgata

20 Amino acid sequence:

MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMFY
 CQQSRKVPWTFGGGTTKLEIKRGGGGSGGGGSGGGGSGVQLKESGPGLVAPSQSLS
 ITCTVSGFSLTGYGVNWRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
 25 KSQVFLKMNSLQTDRTARYYCARGYSNFHYVMDYWGQTSVTVSSDLEPKS
 SDKTHTSPSPAPPELLGGPSVFLFPPPKKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSL
 30 SLSPGKADPSNMDFSRNLYDIGEQLDSEDLASLKFLSLDYTPQRKQEPKDALMLFQ
 RLQEKRMLEESNLSFLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRV
 MLYQISEEVSRSELRSFKFLLQEEISKCKLDDDMNLLDIFIEMEKRVLGEGKLDILK
 RVCAQINKSLKINDYEEFSKERSSSLEGSPDEFSGEELCGVMTISDSPREQDSES
 QTLDKVYQMKSKPRGYCLINNHNFARKAREKVPKLHSIRDRNGTHLDAGALTTTF
 35 EELHFEIKPHDDCTVEQIYEILKIYQLMDHSNMDCFICILSHGDKGIYGTGQEQAP
 IYELTSQFTGLKCPSLAGKPKVFFIQACQGDNYQKGIPVETDSEEQPYLEMDLSSPQ
 TRYIPDEADFLGMATVNNCVSYRNPAEGTWYIQSLCQSLRERCPRGDDILTILTE
 VNYEVSNDKDDKNMGKQMPQPTFLRKKLVFSPD

40

126. 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp8B-TM/CT
 (other caspase 8 isoforms are similar)

Nucleotide sequence:

aagcttatggatttcaagtgacagatttcagcttctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc
 45 aatctccagcttcttggctgtgtcttagtgacagagagccaccatctcctgcagagccagtgaaagtgttgatattatgtcacaagt
 taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
 aggttttagtgagcagtggtctgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtattctgtcagc
 aaagtaggaaggttccttggacgttcggtggaggacccaagctggaatcaaacggggtggcggtggctcgggaggaggtggg
 tcgggtggcgcggtatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
 50 accgtctcaggggttcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
 atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtctt

aaaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggact
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 gcacctgaactcctgggtggatcgtcagtcctccttcccccaaaacccaaggacaccctcatgatctccggacccttgaggtc
 5 acatcgtgggtgggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcc
 aagacaaaagccgcgaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaa
 tggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagc
 cccgagaaccacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtgcaaaag
 gcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctg
 10 gactccgacgggtccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgt
 gatgcatgagggtctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaagcggatccttgaacatggacttca
 gcagaaatctttagatattggggaacaactggacagtgaagatctggcctccctcaagttcctgagcctggactacattccgaaa
 ggaagcaagaacctcatcaaggtgctttagtattccagagactccaggaagagaatgttgaggagaaagcaatctgtccttc
 ctgaaggagctgcttccgaattaatagactggatttgcgtgattacactacaaactagaaaggaggagatggaaagggaactt
 15 cagacaccaggcagggctcaaatctgcctacagggtcatgctctatcagatttcagaagaagtgagcagatcagaattgaggtct
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 gacaatctcggactctcaagagaacaggatagtgaaacacagactttggacaaagttaacaaatgaaaagcaaacctcggggat
 20 actgctgatcatcaacaatcacaattttgaaaagcacgggagaaagtgccaaacttcacagcattaggagacaggaatggaaaca
 cacttggatgcaggggctttgaccacgaccttgaagagcttcatttgagatcaagccccacgatgactgcacagtagagcaaatc
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 catctatggcactgatggacaggaggcccccatctatgagctgacatctcagttcactgggttgaaagtgccttcccttgcgtgaaaa
 cccaaagtgtttttattcaggcttgcagggggataactaccagaaaggtatacctgttgagactgattcagaggagcaaccctattt
 25 agaaatggatttatcatcacctcaaacgagatatacccggatgaggtgactttctgctggggatggccactgtgaataactgtgtt
 cctaccgaaaccctgcagagggaacctggtacatccagtcactttgccagagcctgagagagcgatgtcctcgaggcgatgatatt
 ctaccatcctgactgaagtgaactatgaagtaagcaacaaggtgacaagaaaaacatggggaaacagatgcctcagcctacttt
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30 Amino acid sequence:

MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMFY
 CQQRKVPWTFGGGKLEIKRGGGSGGGGSGGGGSGVQLKESGPGLVAPSQSLS
 35 ITCTVSGFSLTGYGVNWVRQPPGKLEWLGMIWGDGSTDYNSALKSRLSITKDNS
 KSQVFLKMNSLQTDRTARYYCARDGYSNFHYVMDYWGQGTSTVTVSSDLEPKS
 SDKTHTSPSPAPPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHENHNYTQKSL
 40 SLSPGKADPSNMDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQ
 RLQEKRMLEESNLSFLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRV
 MLYQISEEVSRSELRSFKFLQEEISKCKLDDDMNLLDIFIEMEKRVLGEGKLDILK
 RVCAQINKSLLKIINDYEEFSKERSSSLEGSPDEFSGEELCGVMTISDSPREQDSES
 QTLDKVYQMKSKPRGYCLIINNHNFARKAREKVPKLHSIRDNRNGTHLDAGALTTTF
 45 EELHFEIKPHDDCTVEQIYEILKIYQLMDHSNMDCFICILSHGDKGIYGTGQEQAP
 IYELTSQFTGLKCPSLAGKPKVFFIQACQGDNYQKGIPVETDSEEQPYLEMDLSSPQ
 TRYIPDEADFLGMATVNNCVSYRNPAEGTWYIQSLCQSLRERCPRGDDILTILTE
 VNYEVSNNKDDKKNMGKQMPQPTFTLRKKLVFSPD

50

128. 2e12 scFv-hIgG1 (SSS-P)H WCH2 WCH3-hFADD-TM/CT

Nucleotide sequence:

aagcttatggatttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccc
 aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaagtgttgatattatgtcacaagtt
 5 taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
 aggttttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtattctgtcagc
 aaagtaggaaggttccttgagcgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtggg
 tcgggtggcggcggtatcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
 accgtctcagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaaggggtctggagtggtgggaatgat
 10 atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttctt
 aaaaatgaacagctgcgaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggact
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 15 caagacaaagccgcgggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctga
 atggcaaggagtagaagtgcaaggtctccaacaaagccctccagccccatcgagaaaacctctccaaagccaaagggcag
 ccccgagaaccacaggtgtacacctgccccatcccggtgagctgaccaagaaccaggtcagcctgacctgctgtgtaaaa
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 ggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgtctc
 20 gtgatgcagtgaggtctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaagcggatcttgaacccgttct
 ggtgctgctgcactcgggtgtcgtccagcctgtcagcagcgagctgaccgagctcaagttcctatgcctcgggcgcgtgggcaag
 cgaagctggagcgcgtgcagagcggcctagacctcttctcatgtctgtggagcagaacgacctggagccccgggcacaccga
 gctcctgcgcgagctgctgcctcctgcggcgccacgacctgctgcggcgctgcagacttcgaggcggggggcgggcgcc
 gggggcgcgcctggggaagaagacctgtgtgcagcatttaacgtcatatgtgataatgtggggaagattggagaaggtggtc
 25 gtcagctcaaagctcagacaccaagatcgacagcatcaggacagatacccccgcaacctgacagagcgtgtgcgggagtc
 ctgagaatctggaagaacacagagaaggagaacgaacagtgggccacctgggtgggggtctcaggtcctgccagatgaacct
 ggtgggtgacctggtacaagaggttcagcaggccgtgacctccagaacaggagtggggccatgtccccgatgtcatggaactc
 agacgcactctacctccgaagcgtcctgataactcgatgcataacaac

30 Amino acid sequence:

MDFQVQIFSFLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
 CQQRKVPWTFGGGKLEIKRGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSL
 ITCTVSGFSLTGYGVNWVRQPPGKLEWLGMWGDGSTDYNSALKSRLSITKDNS
 35 KSQVFLKMNSLQTDRTARYYCARGYSNFHYVMDYWGQTSVTVSSDLEPKS
 SDKTHTSPSPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTTPVLDSGFFLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSL
 40 SLSPGKADPSNPFLVLLHSVSSSLSSSELTELKFLCLGRVGRKRLERVQSGLDLFSM
 LLEQNDLEPGHTELLRELLASLRRHDLRLRVDDFEAGAAAGAAPGEEDLCAAFNV
 ICDNVGKDWRLARQLKVSDTKIDSIEDRYPRNLTERVRESLRIWKNTKENATV
 AHLVGALRSCQMNLVADLVQEVQQARDLQNRSGAMSPMSWNSDASTSEAS

129. 2e12 scFv-hIgG1 (SSS-P)H P238SCH2 WCH3-hFADD-TM/CT

Nucleotide sequence:

aagcttatggatttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccc
 aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaagtgttgatattatgtcacaagtt
 5 taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
 aggttttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtattctgtcagc
 50 aaagtaggaaggttccttgagcgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtggg

tcgggtggcggcgatctcaggtgcagctgaaggagtcaggacctggcctggcggccctcacagagcctgtccatcacatgc
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 atgggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttctt
 5 aaaaatgaacagctctgcaaaactgatgacacagccagatactactgtgccagagatggttataagtaactttcattactatgttatggact
 actgggggtcaaggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtcccca
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 aagacaaagccgcgggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaa
 10 tggcaaggagtagacaagtgaaggtctcaacaaagccctccagcccccatcgagaaaaccatctccaaagccaaagggcagc
 cccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaag
 gcttctatccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccggtgctg
 gactccgacggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgtccgt
 gatgcatgaggctctgcacaaccactacacgcagaagagcctctcctgtctccgggtaaagcggatcctcgaacctgttctggtg
 15 tgctgctgcactcgggtgtcgtccagcctgtcagcagcgagctgaccgagctcaagttcctatgcctcgggcgctgggcaagcg
 caagctggagcgcgtgcagagcggcctagacctcttctccatgtgctggagcagaacgacctggagcccgggcacaccgagc
 tctgcgcgagctgctgcctccctgcggcgccacgacctgctgcggcgctgcgacttcgaggcggggggcgccggcgccgg
 ggccgcgcctggggaagaagacctgtgtgcagcatttaacgtcatatgtgataatgtggggaagattggagaaggctggctcgt
 cagctcaaagtctcagacaccaagatcagacgcatcaggacagataccccgcaacctgacagagcgtgtgcgggagtcact
 20 gagaatctggaagaacacagagaaggagaacgcaacagtggccacacctgggtgggggctctcaggtcctgccagatgaacctgg
 tggctgacctggtacaagaggttcagcaggcccgtagctccagaacaggagtggggccatgtccccgatgtcatggaactcag
 acgcatctacctccgaagcgtcctgataactcgagatcgataacaac

Amino acid sequence:

25 MDFQVQIFSFLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYP
 CQQSRKVPWTFGGGTKLEIKRGGGSGGGGSGGGGSGVQLKESGPGLVAPSQSL
 ITCTVSGFSLTGYGVNWVRQPPGKLEWLGMIVGDGSTDYNSALKSRLSITKDNS
 30 KSQVFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQTSVTVSSDLEPKS
 SDKTHTSPSPAPPELLGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSFCSVMHEALHNHYTQKSL
 35 SLSPGKADPSNPFLVLLHSVSSSLSSSELTELKFLCLGRVVGKRLKLERVQSGLDLFSM
 LLEQNDLEPGHTELLRELLASLRHDLRLRVDDFEAGAAAGAAPGEEDLCAAFNV
 ICDNVGKDWRLARQLKVS DTKIDSIEDRYPRNLTERVRESLRIWKNTOKENATV
 AHLVGALRSCQMNLVADLVQEVQQARDLQNRSGAMSPMSWNSDASTSEAS

40

130.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-hCD80TM/CT

Nucleotide sequence

45 aagcttatggattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc
 aatctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtt
 taatcgagtgtgaccaacagaaaccaggacagccacccaaactcctcatctctgtgcatccaacgtagaatctgggttcctgcc
 aggtttatgtggcagtggtgtgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgcagc
 aaagtaagcttatggattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgct
 cactcagcttccaacaaccatagctgcatctccaggggagaaggtccatcacctgccgtgccagctccagtgttaagtacatgta
 50 ctggtaccagcagaagtcaggcgccctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcag
 ggcagtggtgtgggaccttattctctcgaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgaggta

5 gttactccgctcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcgggcgggtggtgggtcgggtggc
 ggcggatctcaggtgcagctgaaggaggcaggacctggcctgggtgcaaccgacacagaccctgtccctcacatgcactgtctct
 ggggttcattaccagcagtggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgat
 ggaggcacagattataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagttttctaaaaatcaac
 10 agtctgcaactgatgacacagccatgtattactgtgccagaatccacttgattactggggccaaggagtcaggtcacagtctcct
 ctgatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggaccgtcagttc
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 agcacgtaccgtgtgggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaaca
 15 aagccctcccagccccatcgagaaaacctctccaaagccaaaggcagccccgagaaccacaggtgtacacctgccccca
 tcccggtgatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatgccgtggagtgg
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 ctaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
 agaagagcctctccctgtctccgggtaaagcggatcctcgaacctgctccatcctggccattaccttaatctcagtaaatggaat
 20 tttgtgatatgctgcctgacctactgcttgcaccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtac
 gccctgtataaatcgat

Amino acid sequence:

20 MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLA VSLGQRATISCRASESVEYYVTS
 LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMFY
 CQQSKLMDFQVQIFSLLISASVIMSRGVDIVLTQSPPTIAASPGKEKVTITCRASSVS
 YMYWYQQKSGASPKLWIYDTSKLASGVNRFSGSGSGTSYSLAINTMETEDAATY
 YCQQWSSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTL
 SLTCTVSGFSLTSDGVHWIRQPPGKLEWMGIYYDGGTDYNSAIKSRLSISRDTSK
 25 SQVFLKINSLQTDDTAMYYCARIHFDYWGQGVMTVSSDLEPKSSDKTHTSPSP
 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
 GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP
 VLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKADPS
 30 NLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

131.1D8 scFv mIgAT4-hCD80TM/CT

Nucleotide sequence

35 aagcttatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
 agtctccaacaaccatagctgcattctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
 accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggaggtccaaatcgcttcagtgga
 gtgggtctgggacctcttattctctgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgtagtagtact
 40 ccgctcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcgggcgggtggtgggtcgggtggcggcg
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 caaactgatgacacagccatgtattactgtgccagaatccacttgattactggggccaaggagtcaggtcacagtctcctctgatc
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 ggatgcagtgacagaagaagctgtgcagaattctcgggctgtacagtggtccagcgtctgcctggctgtgctgagcgtgg
 aacagtggcgcattcaagtgcacagttacctatcctgagctgacaccttaactggcacaattgcaaagtcacagtgaacacc
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 ctttaaccctaaagaagtgtggtgcgatggctgcagaaatgaggagctgtcccagaaagctacctagtgtttgagcccctaa
 50 aggagccaggcgaggaggaccacactacgtggtgacaagcgtgttcgtgtatcagctgaaatctggaacagggtgaccagt
 actcctgcatggtgggccacgaggccttgccatgaacttcaccagaagaccatcgaccgtctgtcgggtaaaccaccaatgtc

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 ttttgtgatatgctgcctgacctactgcttggcccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtac
 gccctgtataaatcgatact

5 Amino acid sequence:

MDFQVQIFSLLISASVMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 QKSGASPKLWYDTSKSLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 10 INSLQTTDDTAMYYCARIHFDYWGQGVMTVSSDHICSPPTTPPPSCQPSLSLQRP
 ALEDLLGSDASITCTLNGLRDPEGAVFTWEPSTGKDAVQKKAQVQNSCGCYSVSS
 VLPGAERWNSGASFKCTVTHPESDTLTGTIAKVTVNTFPPQVHLLPPPSEELALN
 ELVSLTCLVRAFPNPKEVLVRWLHGNEELSPESYLVFEPLKEPGEATTYLVTSVLR
 VSAEIWKQGDQYSCMVGHEALPMNFTQKTIDRLSGKPTNVSVSIMSEGEDPSNN
 15 LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

132.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mFADD-TM/CT

Nucleotide sequence

aagcttatggatttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
 20 agtctccaacaaccatagctgcattccaggaggagaaggtcacatcacctgccgtgccagctccagtgtaagttacatgtactggt
 accagcagaagtcaggcgctccctaaactctggatttatgacacatccaagctggcttctggaggtccaaatcgcttcagtgga
 gtgggtctgggacctcttattctctcgcaataacacacatggagactgaagatgctgccacttattactgtcagcagtggaagtagtact
 ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcggcggtgggtgggtcgggtggcggtggcg
 gatctcaggtgcagctgaaggaggcaggacctggcctgggtgaaccgacacagacctgtccctcacatgcactgtctctgggtt
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45 Amino acid sequence:

MDFQVQIFSLLISASVMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 QKSGASPKLWYDTSKSLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 50 INSLQTTDDTAMYYCARIHFDYWGQGVMTVSSDLEPKSSDKTHTSPSPAPPELLG
 GPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP

REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNMDPFL
VLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDLFTVLLEQNDLERGHT
5 GLLRELLASLRHDLLQRLDDFEAGTATAAPPGEADLQVAFDIVCDNVGRDWKR
LARELKVSEAKMDGIEEKYPRSLSERVRESLKVWKNAEKKNASVAGLVKALRTC
RLNLVADLVEEAQESVSKSENMSPLRDSTVSSSETP

133.1D8 scFv hIgG1 (SSS-P)H P238S CH2 WCH3-mFADD-TM/CT

10 Nucleotide sequence

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gtgggtctgggacctcttattctctcgcaataacacatggagactgaagatgctgccacttattactgtcagcagtgtagtagtact
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30 gttcacggtgctgctggagcagaacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggcctcgtgcgccgac
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35 gagcaagagtgagaatatgtcccagttactaagggtattcaactgtgtcttctcagaaacacctgactcgagatcgat

Amino acid sequence:

MDFQVQIFSFLISASVIMSRGVDIVLTQSPTTIAASPGEKVTTICRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
40 SSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTLSTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDETAMYYCARIHFDYWGQGVMTVSSDLEPKSSDKTHTSPSPAPELLG
GSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
45 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNMDPFL
VLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDLFTVLLEQNDLERGHT
GLLRELLASLRHDLLQRLDDFEAGTATAAPPGEADLQVAFDIVCDNVGRDWKR
LARELKVSEAKMDGIEEKYPRSLSERVRESLKVWKNAEKKNASVAGLVKALRTC
50 RLNLVADLVEEAQESVSKSENMSPLRDSTVSSSETP

134.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp3-TM/CT

Nucleotide sequence:

5 aagcttatggattttcaagtcagattttcagcttctgctaatacagtcgttcagtcataatgtccagaggagtcgacattgtgtcactc
 agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
 accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtgga
 gtgggtctgggacctcttattctctcgcaataacaccatggagactgaagatgctgccacttattactgtcagcagtggaagtact
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 gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgactgtctctgggtt
 10 ctcataaccagcgatggtgtacactggattcgacagcctccaggaaagggcttggaatggatgggaataatatattatgatggagg
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 20 ggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagag
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 25 gaagatcatagcaaaaggagcagcttctgtgtgtgattctaagccatggtgatgaaggggtcatttatgggacaaatgggcctgtt
 gaactgaaaagttgactagcttctcagaggcgactactgccggagtctgactggaaagccgaaactcttcatcattcaggcctgc
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 30 ctccctggactccacttccacgaaagaacagatcccgtgtattgtgccatgctcacgaaagaactgtacttttactagctcg
 agatcgatg

Amino acid sequence:

35 MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 INSLQTDDETAMYYCARIHFDYWGGQVMVTVSSDLEPKSSDKTHTSPSPAPPELLG
 GPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 40 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKADPSNMENN
 KTSVDSKSINNFEVKTTHGSKSVDSGIYLDSSYKMDYPEMGICIIINNKNFHKSTGM
 SSRSGTDVDAANLRETFMGLKYQVRNKNDLTREDILELMDSVSKEDHSKRSSFVC
 45 VILSHGDEGVYGTNGPVELKKLTSSFRGDYCRSLTGKPKLFIIQACRGTELDGCIET
 DSGTDEEMACQKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCSMLKLYAH
 KLEFMHILTRVNRKVATEFESFLDSTFHAKKQIPCIVSMLTKELYFYH

135.1D8 scFv hIgG1 (SSS-P)H P238S CH2 WCH3-mcasp3-TM/CT

Nucleotide sequence:

Aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcact
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 5 actccgctcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctgggagggtgggtggcg
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 ggcacagattataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaatcaacagt
 10 ctgcaactgatgacacagccatgtattactgtccagaatccacttggattactggggccaaggagtcaggtcacagtctcctctg
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Amino acid sequence:

MDFQVQIFSLISASVMSRQVDIVLTQSPTTIAASPGKVTITCRASSSVSYMYWY
 QKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 15 SSTPLTFSGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAISKRLSISRDTSKSQVFLK
 INSLQDDTAMYYCARIHFDYWGQGMVTVSSDLEPKSSDKTHTSPSPAPPELLG
 GSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 20 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGKADPSNSNME
 NNKTSVDSKSNINFEVKTIHGSKSVDSGIYLDSSYKMDYPEMGICIIINKNFHKST
 GMSSRSGTDVDAANLRETFMGLKYQVRNKNDLTREDILELMDSVSKEDHSKRSSF
 VCVILSHGDEGVYGTNGPVELKKLTSSFRGDYCRSLTGKPKLFIIQACRGTELDG
 25 IETDSGTDEEMACQKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCSMLKLY
 AHKLEFMHILTRVNRKVATEFESFSLDSTFHAKKQIPCIVSMLTKELYFYH

136.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp8-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
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 ggaagtaacttcagaaaggagtgcctgatgaggcaggcttcgagcaacagaaccacactttagaagtgattcatcatctcaca
 10 gaactatattccggatgaggcagactttctgtctgggaatggctacggatgaagaactgcgttctaccgagatcctgtgaatggaac
 ctggtatattcagtcactttgccagagcctgagggaaagatgtcctcaaggagatgacattcttagcatcctgactggcgtgaactat
 gacgtgagcaataagacgacaggaggaacaagggaaagcagatgccacagcccaccttcacactacggaagaagctcttcttc
 cctccctaagtactcgagatcgatt

15 Amino acid sequence:

MDFQVQIFSLLISASVMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 QKSGASPKLWIYDTSKLASGVNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIAKSRLSISRDTSKSQVFLK
 20 INSLQDDTAMYYCARIHFDYWGQGMVTVSSDLEPKSSDKTHTSPSPAPPELLG
 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKADPSNMDFQS
 25 CLYAIAEELGSEDLAALKFLCLDYIPHKKQETIEDAQKFLRLREKGMLEEGNLSFL
 KELLFHISRWDLLVNFLDCNREEMVRELRPDPAQISPYRVMLFKLSEEVSELELR
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 KNKPRGYCLIINNHDFSKAREDTQLRKMKDRKGTDCDKEALSKTFKELHFEIVSY
 30 DDCTANEIHEILEGYQSADHKNKDCFICILSHGDKGVVYGTGDKKEASTYDLTSYF
 TGSKCPSLSGPKIFFIQACQGSNFQKGVPEAGFEQQNHTLEVDSSSHKNYIPDEA
 DFLGMAATVKNVCVSYRDPVNGTWYIQSLCQSLRERCPQGDDILSILTVNYDVSN
 KDDRRNKGKQMPQPTFTLRKKLFFPP

35 137.1D8 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-mcasp8-TM/CT

Nucleotide sequence:

aagcttatgattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
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 20 cctccctaatgactcgagatcgattc

Amino acid sequence:

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGKEVTITCRASSSVSYMYWY
 QKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 25 SSTPLTFSGGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWRQPPGKGLEWMGITYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 INSLQTTDDTAMYYCARIHFDYWGGQVMVTVSSDLEPKSSDKTHTSPSPAPPELLG
 GSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVS SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 30 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKADPSNMDFQS
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 35 EDYERSSTERRMSLEGREELPPSVLDEMSELKMAELCDSPREQDSESRTSDKVYQM
 KKNKPRGYCLIINNHDFSKAREDTQLRKMKDRKGTDCDKEALSKTFKELHFEIVSY
 DDCTANEIHEILEGYQSADHKNKDCFICCLSHGDKGVVYGTGKEASIDYDLTSYF
 TGSKCPSLSGPKPKIFFIQACQGSNFQKGVPEAGFEQQNHTLEVDSSSHKNYIPDEA
 DFLLGMATVKNCVSYRDPVNGTWYIQSLCQSLRERCPQGDDILSILTG VNYDVSN
 40 KDDRRNKGKQMPQPTFTLRKKLFFPP

138.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcasp3-TM/CT

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Amino acid sequence:

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGKEKVTITCRASSSVSYMYWY
 QQKSGASPKLWIYDTSKSLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFGSGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 25 INSLQTDITAMYYCARIHFDYWGGQVMVTVSSDLEPKSSDKTHTSPSPAPELLG
 GPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKADPSNMENTE
 30 NSVDSKSIKNLEPKIIHGSESMDSGISLDNSYKMDYPEMGLCIINNNKFNHFKSTGMT
 SRSGTDVDAANLRETFRNLYEVRNKNLDTREEIVELMRDVSKEDEHSKRSSFVCV
 LLSHGEEGIIFGTNGPVDLKKITNFFRGDRCSLTGKPKLFIQACRGTELDGCIETD
 SGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCAMLKQYADK
 LEFMHILTRVNRKVATEFESFSFDATFHAKKQIPCIVSMLTKELYFYH

139. 1D8 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp3-TM/CT

Nucleotide sequence:

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15 Amino acid sequence:

MDFQVQIFSLLISASVMSRQVDIVLTQSPTTIAASPGKEVTITCRASSVSVMYWY
 QQKSGASPKLWYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFSGGTKLEIKRGGGSGGGGSGGGGSGVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 20 INSLQTDITAMYYCARIHFDYWGQGVMTVSSDLEPKSSDKTHTSPPSPAPELLG
 GSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKADPSNMENTE
 25 NSVDSKSIKLEPKIHGSEMSDGSISLDNSYKMDYPEMGLCIINNKNFHKSIGMT
 SRSGTDVDAANLRETFRNLYEVRNKNDLTREEIVELMRDVSKEHDSKRSSFVCV
 LLSHGEEGIIFGTNGPVDLKKITNFRGDRCSLTGKPKLFIQACRGTELDGCIETD
 SGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCAMLKQYADK
 LEFMHILTRVNRKVATEFESFSFDATFHAKKQIPCIVSMLTKELYFYH

140.1D8 scFv hIgG1 (SSS-S)H WCH2 WCH3-hcasp8-TM/CT

Nucleotide sequence:

aagcttatggatttcaagtgcagatttccagcttctgtaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactc
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 35 accagcagaagtcaggcgctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtgga
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 cttggcagagcctgagagagcgtatgctctgaggcgatgatattctaccatcctgactgaagtgaactatgaagtaagcaacaa
 15 ggatgacaagaaaaacatggggaacagatgcctcagcctactttcacactaagaaaaaaactgtcttcccttctgattgagcatg
 catcgata

Amino acid sequence:

MDFQVQIFSLLISASVMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 20 QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFSGGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGITYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 INSLQTTDDTAMYYCARIHFDYWGGQVMVTVSSDLEPKSSDKTHTSPSPAPELLG
 GPSVFLFPPKPKDTLMISRTPVETCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 25 REEQYNSTYRVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFCFVMHEALHNHYTQKSLSLSPGKADPSNMDFSR
 NLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPKDALMLFQRLQEKRMLEESNLSFL
 KELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRVMYQISEEVSRLS
 30 FKFLQEEISKCKLDDDMNLLDIFIEMEKRVLGEGKLDILKRVCAQINKSLKIND
 YEEFSKERSSSLEGSPDEFSGEELCGVMTISDSPREQDSESQTLTKVYQMKSKPR
 GYCLINNHNFKAAREKVPKLHSIRDNRNGTHLDAGALTTTFFELHFEIKPHDDCTV
 EQIYEILKIYQLMDHSNMDCFICILSHGDKGIYGTGQEAPIYELTSQFTGLKCPS
 LAGKPKVFFIQACQGDNYQKGIPVETDSEEQPYLEMDLSSPQTRYIPDEADFLGM
 35 ATVNNCVSYRNP AEGTWYIQLCQSLRERCPRGDDILTILTEVNYEVS NKDDKKN
 MGKQMPQPTFTLRKKLVFSPD

141.1D8 scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hcasp8-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
 agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagttacatgtactggt
 accagcagaagtcaggcgccctcccctaaactctgatttatgacacatccaagctggcttctggagtccaatcgcttcagtggca
 gtgggtctgggaccttattctctcgaatcaacaccatggagactgaagatgctgccactattactgtcagcagtgagtagtact
 ccgctcacgttcgggtctgggaccaagctggagatcaaacgggggtggcggtggctcgggcgggtgggtgggtcgggtggcg
 45 gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcatgactgtctctgggtt
 ctcatataaccagcgatggtgtacactggattcagagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
 cacagattataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaatcaacagctctg
 caaactgatgacacagccatgtattactgtgccagaatccacttggattactggggccaaggagtcaggtcacagtctcctctgatct
 ggagcccaaatcttctgacaaaactcacacatcccaccgtcccagcacctgaactcctgggtggatcgtcagcttctcttccc
 50 cccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtgggtgacgtgagccacgaagacctga
 ggtcaagttcaactggtacgtggacggcggtggaggtgcataatgccaagacaagccgcgggaggagcagtacaacagcacgt

accgtgtggctcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctccaacaaagccct
 cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccggg
 atgagctgaccaagaaccagggtcagcctgacctgctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagca
 atgggcagccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttctctacagcaagctcaccgt
 5 ggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacgcagaagag
 cctctccctgtctccgggtaaagcggatccttcgaacatggacttcagcagaaatctttatgatattggggaacaactggacagtga
 gatctggcctccctcaagttcctgagcctggactacattccgcaaaaggaagcaagaacccatcaaggatgccttgatgttattccag
 agactccaggaaaagagaatgttggaggaaagcaatctgtccttctgaaggagctgctcttccgaattaatagactggatttgctg
 attacctacctaataactagaaaggaggagatggaaagggaaacttcagacaccaggcagggctcaaatttctgcctacagggtca
 10 tgctctatcagatttcagaagaagtgagcagatcagaattgaggtcttttaagttcttttgaagaggaaatctccaaatgcaaactgg
 atgatgacatgaacctgctggatatttcatagagatggagaagagggatcctctgggagaaggaaagttggacatcctgaaaaga
 gtctgtgccccaaatcaacaagcctgctgaagataatcaacgactatgaagaattcagcaaagagagaagcagcagcctgaag
 gaagtctgatgaatttcaaatggggaggagttgtgtgggtaatgacaatctcgactctccaagagaacaggatagtgaatcac
 agactttggacaaagtttaccaaatgaaaagcaaacctcggggatactgtctgatcatcaacaatcacaatttgcaaaagcacggg
 15 agaaagtgcccaaacttcacagcattagggacaggaatggaacacacttgatgcaggggcttgaccacgaccttgaagagctt
 cattttgagatcaagccccacgatgactgcacagtagagcaaatctatgagatttgaaaatctaccaactcatggaccacagtaaca
 tggactgcttcatctgctgtatctctccatggagacaagggcatcatctatggcactgatggacaggaggccccatctatgagct
 gacatctcagttcactggttgaagtgccttcccttgcctggaacccaaagtgtttttatcaggcttgtaggggataactacca
 gaaaggtatacctgttgagactgattcagaggagcaacctatttagaaatggatttatcatcacctcaaagagatatatccggat
 20 gaggtgactttctgctgggagtgccactgtgaataactgtgttctaccgaaacctgcagagggaacctggtacatccagtca
 ctttgccagagcctgagagagcgatgtcctcgaggcgatgatattctaccatcctgactgaagtgaactatgaagtaagcaacaa
 ggatgacaagaaaaacatggggaaacagatgcctcagcctacttccactaagaaaaaaacttgcttcccttctgattgagcatg
 catcgataa

Amino acid sequence:

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
 QQKSGASPKLWIYDTSKLASGVNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
 SSTPLTFSGGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSTCTV
 SGFSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
 30 INSLQTTDDTAMYYCARIHFDYWGQGVMTVSSDLEPKSSDKTHTSPSPAPELLG
 GSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGKADPSNMDFSR
 35 NLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPKDALMLFQRLQEKRMLEESNLSFL
 KELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRVMLYQISEEVSRSELRS
 FKFLQEEISKCKLDDDMNLLDIFIEMEKRVILGEGKLDILKRVCAQINKSLLKIIND
 YEEFSKERSSSLEGSPDEFSGEELCGVMTISDSPREQDSESQTLKVKYQMKSKPR
 GYCLIINHNHFAKAREKVPKLHSIRDRNGTHLDAGALTTTFEELHFEIKPHDDCTV
 40 EQIYEILKIYQLMDHSNMDCFICCLSHGDKGIYGTGQEAPIYELTSQFTGLKCPS
 LAGKPKVFFIQACQGDNYQKGIPVETDSEEQPYLEMDLSSPQTRYIPDEADFLGGM
 ATVNNCVSYRNP AEGTWYIQSLCQSLRERCPRGDDILTILTEVNYEVSNKDDKKN
 MGKQMPQPTFTLRKKLVFSPD

145. hCTLA4 IgAH IgACH2CH3

Nucleotide sequence:

atggcttgacctggaatttcagcggcacaaggctcagctgaacctggctgccaggacctggccctgcactctcctgtttttcttctcttc
 atccctgtcttctgcaaagcaatgcacgtggcccagcctgctgtgtgactggccagcagccgaggcatcgccagcttctgtgtga
 gtatgcatctccaggcaaagccactgaggtccgggtgacagtgtctcgccaggctgacagccaggtgactgaagtctgtcggc
 50 aacctacatgacggggaatgagttgaccttctagatgattcatctgcacgggcacctccagtggaaatcaagtgaacctcactat
 ccaaggactgaggggccatggacacgggactctacatctgcaaggtggagctcatgtaccaccgccatactacctgggcatagg

caacggaacccagatttatgtaattgatccagaaccgtgccagattctgatcagccagttccctcaactccacctaccccatctccc
 tcaactccacctaccccatctccctcatgctgccaccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttca
 gaagcgatctcacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagc
 gctgttcaaggaccacctgacctgtgacctgtggtgctacagcgtgtccagtgctcctgccgggctgtgccgagccatggaacca
 5 tgggaagaccttcacttgactgtgcctaccccgagtccaagaccccgtaaccgccaccctctcaaatccggaacacattcc
 ggcccagggtccacctgctgccgccgctcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggc
 ttcagccccaaggatgtgctggtcgtggtgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatccggg
 caggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggaca
 ccttctctgcatggtggggccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacca
 10 tgtcaatgtgtctgttgcacatggcggaggtggacggcacctgctactgataatctaga

Amino acid sequence:

MACLGFORHKAQLNLAARTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIAS
 FVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMTGNELTFLDDSICTGTSSGN
 15 QVNLTIQGLRAMDTGLYICKVELMYPPPYLYLGINGTQIYVIDPEPCPDSDQVPVST
 PPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWPS
 SGKSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLS
 KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKY
 LTWASRQEPSQGTTFFAVTSILRVAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
 20 LAGKPTHVNVSVVMAEVDGTCY

146. hCTLA4 IgA WH WCH2 T4CH3 (hCTLA4 IgAH IgACH2CH3)

Nucleotide sequence:

atggcttgcccttgatttcagcggcacaaggctcagctgaacctggctgccaggacctggccctgcactctctgtttttctctcttc
 25 atccctgtctctgcaaaagcaatgcacgtggccagcctgctgtggtactggccagcagccgaggcatcgccagcttgtgtgga
 gtatgcatctccaggcaaaagccactgaggtccgggtgacagtgtctcggcaggctgacagccaggtgactgaagtctgtcgggc
 aacctacatgacggggaatgagttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctactat
 ccaaggactgagggccatggacacgggactctacatctgcaaggtggagctcatgtaccaccgccatactacctgggcatagg
 caacggaacccagatttatgtaattgatccagaaccgtgccagattctgatcagccagttccctcaactccacctaccccatctccc
 30 tcaactccacctaccccatctccctcatgctgccaccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttca
 gaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagc
 gctgttcaaggaccacctgacctgtggtgctgtacagcgtgtccagtgctcctgccgggctgtgccgagccatggaacca
 tgggaagaccttcacttgactgtgcctaccccgagtccaagaccccgtaaccgccaccctctcaaatccggaacacattcc
 ggcccagggtccacctgctgccgccgctcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggc
 35 ttcagccccaaggatgtgctggttcgctggtgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatccggg
 caggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggaca
 ccttctctgcatggtggggccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacca
 tgtcaatgtgtctgttgcacatggcggaggtggactgataatctaga

Amino acid sequence:

MACLGFORHKAQLNLAARTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIAS
 FVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMTGNELTFLDDSICTGTSSGN
 QVNLTIQGLRAMDTGLYICKVELMYPPPYLYLGINGTQIYVIDPEPCPDSDQVPVST
 PPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWPS
 45 SGKSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLS
 KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKY
 LTWASRQEPSQGTTFFAVTSILRVAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
 LAGKPTHVNVSVVMAEVD

147. hIGA WH WCH2 T18CH3

Nucleotide sequence:

5

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15

20

25

40

45

KSLSLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVR
PV

HCD16lowFL+NL

5 Nucleotide sequence:

aagcttgccgccatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgaggactgaagatctcccaaagg
ctgtggtgttcctggagcctcaatggtacagggtgctcgagaaggacagtgactctgaagtgccaggaggcctactcccctgag
gacaattccacacagtggttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgctgccacagtcgacgac
10 agtggagagtacagggtgccagacaaacctccaccctcagtgaccgggtgcagctagaagtccatcggctggctgttctcca
ggccccctcggtgggtgttcaaggaggaagaccctattcacctgaggtgtcacagctggagaacactgctctgcataaggtcacat
attacagaatggcaaaggcaggaagtatcttcataaattctgacttctacattccaaaagccacactcaagacagcggtcctac
ttctgcagggggctgttgggagtaaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggttggcagtgtaaccatctc
atcattcttccacctgggtaccaagtccttctgcttgggtatggactccttttgcagtgagacacaggactatattctctgtgaaga
15 caaacattcgaagctcaacaagagactggaaggaccataaatttaaatggagaaaggaccctcaagacaaatgacct

15

Amino acid sequence:

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPE
DNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLL
LQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATL
20 KDSGSYFCRGLVGSKNVSETVNITITQGLAVSTISSFFPPGYQVSFCLVMVLLFAV
DTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK

From the foregoing, it will be appreciated that, although specific
embodiments of the invention have been described herein for the purpose of illustration,
25 various modifications may be made without deviating from the spirit and scope of the
invention. Accordingly, the present invention is not limited except as by the appended
claims.

All patents, patent applications, publications, scientific articles, web sites, and other
documents and materials referenced or mentioned herein are indicative of the levels of skill
30 of those skilled in the art to which the invention pertains, and each such referenced
document and material is hereby incorporated by reference to the same extent as if it had
been incorporated by reference in its entirety individually or set forth herein in its entirety.
Additionally, all claims in this application, and all priority applications, including but not
limited to original claims, are hereby incorporated in their entirety into, and form a part of,
35 the written description of the invention. Applicants reserve the right to physically
incorporate into this specification any and all materials and information from any such
patents, applications, publications, scientific articles, web sites, electronically available
information, and other referenced materials or documents. Applicants reserve the right to
physically incorporate into any part of this document, including any part of the written
40 description, the claims referred to above including but not limited to any original claims.

The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential.

Thus, for example, in each instance herein, in embodiments or examples of the present invention, any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms in the specification. Also, the terms "comprising", "including", "containing", *etc.* are to be read expansively and without limitation. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. It is also that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality (for example, a culture or population) of such host cells, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art,

and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

5 The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein. For example, the subject matter of the instant invention may optionally exclude any of the subject matter or sequences described or included in related application published as U.S.S.N. 2003/0118592 A1 on 10 June 26, 2003 (and the sequence listings of SEQ ID NOS: 1-427 published by USPTO), by Ledbetter et al. entitled "Binding Domain-Immunoglobulin Fusion Proteins".

Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual 15 member or subgroup of members of the Markush group.

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